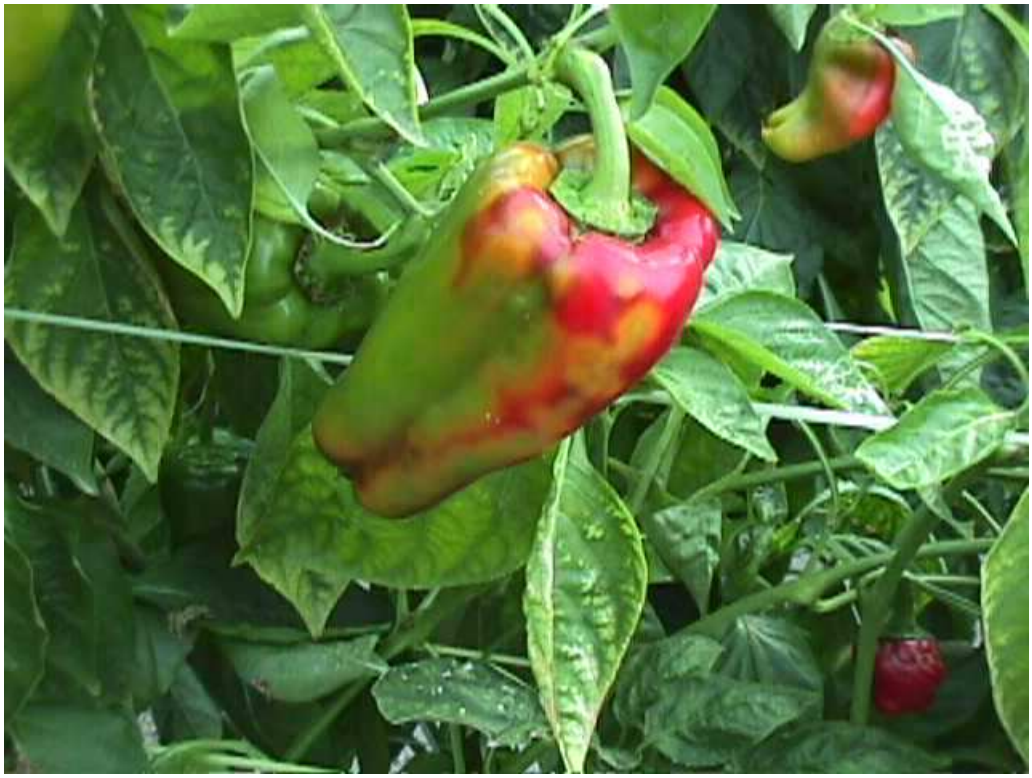
VIIth INTERNATIONAL PLANT VIRUS EPIDEMIOLOGY SYMPOSIUM

Plant Virus Epidemiology: Current status and future prospects



AGUADULCE (ALMERIA), SPAIN, APRIL 11 - 16, 1999

INTERNATIONAL SOCIETY OF PLANT PATHOLOGY

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

INSTITUTO NACIONAL DE INVESTIGACION Y TECNOLOGIA AGRARIA Y ALIMENTARIA

UNIVERSIDAD DE ALMERIA

DIPUTACION DE LA PROVINCIA DE ALMERIA

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Plant Virus Epidemiology: Current status and future prospects

ABSTRACTS

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PLANT VIRUS EPIDEMIOLOGY:
CURRENT STATUS AND FUTURE PROSPECTS

VIIth INTERNATIONAL PLANT VIRUS EPIDEMIOLOGY SYMPOSIUM

AGUADULCE (ALMERIA), SPAIN. APRIL, 11 – 16, 1999

These Symposia are organized under the auspices of the Plant Virus Epidemiology Committee of the International Society of Plant Pathology

Previous Symposia were held in:

Oxford, UK, 28-31 July 1981

Australia, 25-27 August 1983

Orlando, USA, 6-8 August 1986

Montpellier, France, 1-5 September 1989

Valenzano (Bari), Italy, 27-31 July 1992

Maale Ha`Chamisha (Jerusalem), Israel, 23-28 April 1995

The photograph on the front page illustrates typical symptoms of a pepper fruit infected with Tomato Spotted Wilt Tospovirus. It was taken by Dr. A. Fereres during a visit of the Organizing Committee to the Almeria greeenhouses in March 1998.

PARTICIPATING ORGANIZATIONS AND SPONSORS

* INTERNATIONAL SOCIETY OF PLANT PATHOLOGY
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* SPANISH INSTITUTE OF AGRICULTURAL RESEARCH (INIA)
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* SEMINIS VEGETABLE SEEDS
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* FUNDACION PARA LA INVESTIGACION AGRICOLA DE LA PROVINCIA DE ALMERIA (F.I.A.P.A.)
* DIPUTACION DE LA PROVINCIA DE ALMERIA

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Ms. P. Gonzalez (CSIC, Madrid)
Ms. Y. Rieche (CSIC, Madrid)

PROGRAMME

SUNDAY, APRIL 11

17:00 – 20:00 Registration

20:00 – 22:00 Welcome Reception

MONDAY, APRIL 12

MORNING

8:30 – 9:30 Registration

9:30 – 10:00 Welcome and Opening Session

10:00 – 10:45 **Chairman's opening address. J. M. Thresh**

The past 100 years in plant virus epidemiology and the continuing ecological tradition. The role of the ISPP Epidemiology Committee.

10:45 – 11:00 General Dicussion

11:00 – 11:30 Coffee Break

11:30 – 13:30 ***SESSION 1: Transmission mechanisms of plant viruses***

Chairs: T. P. Pirone, B. Raccah and D. Peters

ORAL PRESENTATIONS:

11:30 – 11:45 Electrical analysis of non-circulative virus transmission*:* **A. Fereres** and J.L. Collar.

11:45 – 12:00 Potyvirus transmission by aphids: virion-helper component-stylet interactions: **T.P. Pirone** and R Y. Wang.

12:00 – 12:15 Mediated transmission of zucchini yellow mosaic virus and other potyviruses using helpers purified by the Ni^{2+}-NTA resin: **B. Raccah**, D. Kadouri, H. Huet, Y-H. Peng and A. Gal-On

12:15 – 12:30 Molecular mechanisms of cauliflower mosaic virus aphid transmission: unravelling increasing complexity: **S. Blanc**, E. Hebrard, R. Froissart and M. Drucker.

12:30 – 12:45 Geminiviruses: intracellular pathway and transport sites in their insect vectors: **V. Medina**, K. Theodoridis and P.G. Markham.

12:45 – 13:00 Coat protein gene replacement results in whitefly-transmission of an insect non-transmissible geminivirus isolate: P. Höfer, M. Höhnle, I D. Bedford, P.G. Markham and **T. Frischmuth**.

13:00 – 13:15 Sex-mediated transmission and propagation of tomato yellow leaf curl geminivirus by whiteflies: **H. Czosnek** and M. Ghanim.

13:15 – 13:30 Infection of transmitting and non-transmitting thrips by tomato spotted wilt virus: Nagata, R. Goldbach and **D. Peters**.

POSTER PRESENTATIONS:

P-S1-1- Plum pox virus helper component: molecular interactions with coat protein and synergy with potato virus X: S. Yang and M. Ravelonandro.

P-S1-2- Studying the genetics of virus transmission: development of genetic systems for leafhoppers: K. Theodorides, A. Secker, V. Medina, I. Bedford and P.G. Markham

P-S1-3- Aphid transmission and long-term absence of sweetpotato feathery mottle virus in sweetpotato: T. Alicai, V. Aritua and R.W. Gibson.

P-S1-4- Vectorial variability among population of *Frankliniella occidentalis* populations transmitting tomato spotted wilt virus and the pattern of virus spread in lettuce fields in Israel: A. Kritzman, N. Ganaim, A. Gera and B. Raccah.

P-S1-5- Transmission of tomato spotted wilt tospovirus by *Thrips tabaci* populations and determination of the median latent period: E.K. Chatzivassiliou, N.I. Katis and D. Peters.

P-S1-6- Mineral oil effect on *Myzus persicae* probing behavior and subsequent potato virus Y acquisition from pepper plants: J.L. Collar and A. Fereres.

P-S1-7- Correlation between whitefly feeding behavior and tomato yellow leaf curl virus transmission: Y.X. Jiang, C. de Blas, L. Barrios and A. Fereres.

P-S1-8- Helper component mutations in two non-conserved residues are associated with lack of aphid transmissibility of a pepper potato virus Y isolate: C. Llave, B. Martínez-García, P. González-Jara, J.R. Díaz-Ruíz and D. López-Abella.

P-S1-9- Potyvirus helper component gene replacement: effects on infectivity and aphid transmissibility: B. Martínez-García, C. Llave, M.R. Fernández, J.J. López-Moya, J.A. García, T. Orero, J.R. Díaz-Ruiz and D. López-Abella.

AFTERNOON

13:30 – 15:30 Lunch and Poster Inspection

15:30 – 17:30 ***SESSION 2: Current approaches to plant virus epidemiology***

Chairs: F. Garcia-Arenal, R. Hull and C. L. Niblett

ORAL PRESENTATIONS:

15:30 – 15:45 Non-persistent and semi-persistent virus detection in single aphids by squash capture-PCR: **O. Esteban**, A. Olmos, C. Marroquín, C. Varveri, A. Hermoso de Mendoza, M.T. Gorris and M. Cambra.

15:45 – 16:00 Detection and differentiation of strains of citrus tristeza virus being transmitted in Florida since the establishment of *Toxoptera citrida* (Kirkaldy), the brown citrus aphid: H. Genc, K.L. Manjunath, S. Halbert, L. Brown, B. Cevik, R F. Lee and **C.L. Niblett**.

16:00 – 16:15 Analysis of the geographical distribution of cotton leaf curl disease associated begomoviruses in Pakistan: S. Mansoor, M. Hassain, S.H. Khan, A. Bashir, Y. Yusuf, K.A. Malik, R.W. Briddon and **P.G. Markham**.

16:15 – 16:30 Genetic diversity of rice tungro spherical virus in tungro-endemic provinces of the Philippines and Indonesia: **O. Azzam**, Ma L.M. Yambao, M. Muhsin, K.L. McNally and K.M.L. Umadhay.

16:30 – 16:45 Molecular epidemiology of cucumber mosaic virus and its satellite RNA: **F. García-Arenal**, A. Fraile, J.M. Malpica and F. Escriu.

16:45 – 17:00 Biodiversity of populations of cucumber mosaic virus in Italy before and after virus outbreaks in 1988: M.M. Finetti Sialer, F. Cillo, F. Paradies and **D. Gallitelli**.

17:00 – 17:15 Variation among both aphid clones and virus isolates regulate potato leafroll luteovirus transmission by vectors of the *Myzus persicae* group: **D. Bourdin**, L. Terradot, J.C. Simon, J. Rouzé, S. Tanguy, N. Leterme and Y. Robert.

17:15 – 17:30 Biological and molecular characterization of clones of *Myzus persicae* and *Myzus antirrhinii* with different efficiencies for potato leafroll virus transmission: **J.A.T. Woodford** and B. Fenton.

POSTER PRESENTATIONS:

P-S2-1- First report of tobacco mild green mosaic tobamovirus in eggplant and pepper crops in Spain: M. Luis-Arteaga, E. Saez , B. Berdiales and E. Rodríguez-Cerezo.

P-S2-2- Genetic diversity and BYDV-transmission efficiency of *Rhopalosiphum padi* genotypes: H–U. Leistner, A. Habekuß, E. Schliephake and G. Proeseler.

P-S2-3- Serological, molecular and biological variabilities of rice yellow mottle virus isolates from Ivory Coast: P. N'Guessan, A. Pinel, M.L. Caruana, R. Fructos, A. Sy, A. Ghesquiere and D. Fargette.

P-S2-4- Epidemiological factors that determine the evolution of the satellite RNA of cucumber mosaic virus: A. Fraile, F. García-Arenal and F. Escriu.

P-S2-5- Nucleotide sequence of the 3'-terminal region of an isolate of potato virus Y inducing veinal necrosis in pepper (PVYnnp): A. Crescenzi, A. Fanigliulo, S. Comes, M. Nuzzaci and P. Piazzolla

P-S2-6- *Myzus cerasi* vector of the sweet cherry strain of plum pox potyvirus : A. Crescenzi, S. Comes, A. Fanigliulo, M. Nuzzaci and P. Piazzolla.

P-S2-7- Genetic characterization of tobacco potato virus Y strains in Spain: A. Romero and F. Ponz.

P-S2-8- Towards the establishment of the genetic strains of turnip mosaic virus: F. Sánchez, C. Jenner, J. Walsh and F. Ponz.

P-S2-9- Natural occurrence of cucumber mosaic cucumovirus and its satellite RNA on pepper crops in Argentina: F.A. Atencio, O. Gracia, R.O. Zandomeni and O. Grau.

P-S2-10- Improvement of diagnostic methods for the detection of viruses and viroids of phytosanitary importance: M. Barba, F. Faggioli, G. Pasquini and L. Tomassoli.

P-S2-11- Analysis of the incidence of maize-infecting potyviruses in middle Germany: E. Fuchs, M. Grüntzing, F. Hohmann and U. Oertel.

P-S2-12- Development of an isothermal amplification method to distinguish cucumber mosaic virus isolates: C. de Blas, M. Klerks, C. Schoen, G. Carazo and G. Leone.

P-S2-13- Epidemiology and genetic diversity of bean pod mottle comovirus: S.A. Ghabrial and A.J. Clark.

P-S2-14- The use of polyclonal and monoclonal antibodies directed against potyvirus Y and insitu detection of this virus on tobacco leaf epidermis: S. Rouis, R. Gargouri-Bouzid and H. Ayadi.

17:30 – 18:00 Coffee Break

18:00 – 19:00 Discussion of Sessions 1 & 2 and Posters

TUESDAY, APRIL 13

MORNING

9:00 – 11:00 ***SESSION 3: Whitefly-associated problems of vegetable crops***

Chairs: E. Moriones, J. K. Brown and G. C. Wisler

ORAL PRESENTATIONS:

9:00 – 9:15 Ocurrence of cucurbit yellow stunting disorder virus (CYSDV) and beet pseudo-yellows virus in cucurbit crops in Spain and transmission of CYSDV by two biotypes of *Bemisia tabaci*: B. Berdiales, J.J. Bernal, E. Sáez, B. Woudt, F. Beitia and **E. Rodriguez-Cerezo**.

9:15 – 9:30 Whitefly-transmitted viruses infecting sweetpotato in The United States: **R.A. Valverde**.

9:30 – 9:45 Expansion of tomato-infecting criniviruses into new areas: **G.C. Wisler**, JE. Duffus and H-Y. Liu.

9:45 – 10:00 Relative importance of tomato yellow leaf curl virus-Is and –Sr species in infections of tomato in Spain: S. Sánchez-Campos, J.A. Díaz, C. Soria, R. Camero and **E. Moriones**.

10:00 – 10:15 Management of geminivirus epidemics of field-grown tomato in Florida and The Dominican Republic: **P.A. Stansly**.

10:15 – 10:30 The epidemiology and management of tomato leaf curl virus and *Bemisia tabaci* in southern India: **J. Colvin**, V. Muniyappa, H.K. Ramappa, A.K. Cherian, H.M. Venkatesh, N. Nagaraju, M.N. Maruthi and S.K. Green.

10:30 – 10:45 Tomato breeding lines derived from *Lycopersicon hirsutum* that are inmune and tolerant to tomato yellow leaf curl virus (TYLCV): S. Vidavsky and **H. Czosnek**.

10:45 – 11:00 The effect of tomato yellow leaf curl virus on new breeding lines with high levels of resistance to the virus: **M. Lapidot**, M. Friedmann, S. Cohen and M. Pilowsky.

11:00 – 11:30 Coffee Break

11:30 – 13:30 ***SESSION 3*** **(CONT.)**

11:30 – 11:45 Progress in the diagnosis and epidemiological characterisation of cassava mosaic geminiviruses in East Africa: **J.P. Legg** and G. Okao-Okuja.

11:45 – 12:00 Geminiviruses and cassava whiteflies across Africa: R.W. Briddon, J.A. Farquhar, C. Roussot, G.K. Banks, I.D. Bedford, J. Legg and **P.G. Markham**.

12:00 – 12:15 Factors driving the current epidemic of severe cassava mosaic disease in East Africa: **J. Colvin**, G.W. Otim-Nape, J. Holt, C. Omongo, S. Seal, P. Stevenson, G. Gibson, R.J. Cooter and J.M. Thresh.

12:15 – 12:30 The ecology and dissemination of whitefly-transmitted viruses in Latin America: **F.J. Morales** and J. Klass.

12:30 – 12:45 Genetic diversity among geminiviruses infecting crops and weeds in Jamaica: **M.E. Roye**, W.A. McLaughlin, M.K. Nakhla and D.P. Maxwell.

12:45 – 13:00 Ecologically oriented management strategies for *Bemisia* in agricultural systems: **T.J. Henneberry**.

13:00 – 13:15 The mitochondria COI gene as an informative molecular marker for phylogenetic analysis and identification of *Bemisia tabaci* (Genn.), the whitefly vector of geminiviruses: **J.K. Brown** and I. Torres-Jerez.

13:15 – 13:30 The core region of the coat protein gene of viruses of the *Geminiviridae* is phylogenetically informative: introducing an interactive website for begomovirus identification using the core CP sequence: **J.K. Brown**, A.M. Idris, I. Torres-Jerez and S.D. Wyatt.

POSTER PRESENTATIONS:

P-S3-1- Variation in host response to *Bemisia tabaci* (Homoptera: Aleyrodidae) in tomato plants: M. Muñiz, G. Nombela and F. Beitia.

P-S3-2- Settling behavior of *Bemisia tabaci* (Homoptera: Aleyrodidae) on some common weeds: M. Muñiz and Y. Rieche.

P-S3-3- Identification of some common weeds as reservoirs for tomato yellow leaf curl virus transmitted by *Bemisia tabaci* (Gennadius): Y.X. Jiang, C. De Blas and M. Muñiz.

P-S3-4- Laboratory evidence of interbreeding between biotypes of *Bemisia tabaci* (Homoptera, Aleyrodidae) present in Spain: M. Ronda, A. Adán, D. Cifuentes, J.L. Cenis and F. Beitia.

P-S3-5- Genetic relationship of biotypes of *Bemisia tabaci* (Homoptera:Aleyrodidae) present in Spain based on RAPDS and AFLPS: P. Guirao, M.T. Cervera, A. Moya, J.A. Cabezas, F. Beitia, J.M. Martínez-Zapater and J.L. Cenis.

P-S3-6- Weeds as reservoirs of tomato yellow leaf curl viruses in Spain: S. Sánchez-Campos, J. Navas-Castillo, J.A. Díaz, J. Reina, E.R. Bejarano and E. Moriones.

P-S3-7- The use of nets and insecticide treatments in the control on *Bemisia tabaci* Genn. populations and tomato yellow leaf curl virus spread in protected tomato crops in Portugal: A.F. Arsénio, E. Neto,

N. Ramos, S. Mangerico, J.E. Fernandes, A.M.P. Lavadinho, A. Lopes, J.M. Guimarães and D. Louro.

P-S3-8- The effect of varietal mixtures on the progress of cassava mosaic virus disease grown under epidemic conditions in Uganda: W.S. Sserubombwe, J.M. Thresh, G.W. Otim-Nape and D.S.O. Osiru.

P-S3-9- An overview of the incidence of cassava mosaic disease in East Africa, 1998 update: P. Sseruwagi, J.P. Legg and G.W. Otim-Nape.

P-S3-10- The geographic distribution of cassava *Bemisia tabaci* biotypes in relation to the current epidemic of cassava mosaic disease in East Africa: M.N. Maruthi, J. Colvin and S. Seal.

P-S3-11- Integrating various neonicotinoid insecticides into chemical control practices for sustainable whitefly management: N. Prabhaker, N.C. Toscano and T.J. Henneberry.

AFTERNOON

13:30 – 15:30 Lunch and Poster Inspection

15:30 – 16:30 Discussion of Session 3 and Posters

16:30 – 17:30 ***Introduction to the Field Trip***. **Chair: T. Cabello**

Vegetables production systems in Almeria greenhouses:

- Agronomic practices (M. Gallardo)
- Virological aspects (C. Jorda)
- Integrated production (J. Belda)

17:30 – 18:00 Coffee Break

18:00 – 19:00 **Open meeting: activities and future of the ISPP Plant Virus Epidemiology Committee.**

WEDNESDAY, APRIL 14

MORNING

9:00 – 14:00 FIELD TRIP

A visit to "El Ejido" greenhouses including integrated production cooperatives, vegetable processing and export companies.

AFTERNOON

14:00 – 16:00 Lunch

16:00 – 22:00 ALMERIA TOUR

Visit to the historic monuments and free time for shopping and dinner. Transportation and admission to the monuments but not dinner are included in the registration fee.

OR

EXCURSION TO CABO DE GATA-NIJAR NATURE RESERVE

Transportation and visit to the Nature Reserve located 40 km east of Almeria.

THURSDAY, APRIL 15

MORNING

9:00 – 11:00 ***SESSION 4: Modelling plant virus epidemics***

Chairs: M. Jeger and L. Madden

ORAL PRESENTATIONS:

9:00 – 9:15 A theoretical assessment of vector-virus transmission mechanisms on plant virus disease epidemics**: L.V. Madden**, M.J. Jeger and F. van den Bosch.

9:15 – 9:30 A new general model of plant-virus disease infection which incorporates vector aggregation: **J. Holt**, X.S. Zhang and J. Colvin.

9:30 – 9:45 Modelling plant virus epidemics in a combined field-nursery system: M.J. **Jeger,** M. Y. Dutmer and F. van den Bosch.

9:45 – 10:00 The influence of cultivation practices on the spatial distribution of viruses in Australian hop gardens: **S.J. Pethybridge**, C.R. Wilson, F.J. Ferrandino and G.W. Leggett.

10:00 – 10:15 Regional analysis of plant virus epidemics with geographic information systems and geostatistics**: M.R. Nelson** and T.V. Orum.

10:15 – 10:30 Use of a mathematical model as an analytical tool to prioritize IPM research and interventions for whitefly-transmitted geminiviruses in Latin America: **P.K. Anderson**.

10:15 – 10:30 Epidemiology of two phytoplasm strains associated with yellow crinkle in papaya**: F.W. Nutter**, Jr., A.C. Padovan and K.S. Gibb**.**

10:45 – 11:00 Forecasting aphid outbreaks and spread of cucumber mosaic virus in *Lupinus* - a simulation model for a mediterranean type climate: **D.J. Thackray**, A.J. Diggle. F.A. Berlandier and R.A.C. Jones.

POSTER PRESENTATIONS:

P-S4-1- Modelling vector dynamics and the spread of insect-transmitted viruses: D. Morgan, I. Barker and K.A. Walters.

P-S4-2- Temporal and spatial patterns of spread with the necrotic and non-necrotic strains of bean yellow mosaic virus in narrow-leafed lupin: Y. Cheng and R.A.C. Jones.

P-S4-3- Temporal and spatial patterns of spread of cucumber mosaic virus in chickpea: S.J. McKirdy and R.A.C. Jones.

P-S4-4- Dynamic models of host-plant infection by helper-dependent virus complexes: X.S. Zhang, J. Holt and J. Colvin

P-S4-5- SMPVY: a simulation model for potato virus Y infecting pepper (*Capsicum annuum* L) crops**:** J. Sánchez-Ponz and A. Fereres.

P-S4-6- Spread of potato virus Y in potato from an external source: spatial and temporal patterns**:** F.J. Legorburu, R. Marquínez and J.A. Ruiz de Gauna.

P-S4-7- Total aphid flight monitoring by yellow water traps: steps towards modelling: M. González de Murillo, M.D. Ramos-Pérez and F.J. Legorburu.

P-S4-8- Modeling virus epidemic development using long-term statistics: F.P. Demyanenko.

P-S4-9- Temporal and spatial spread of tomato spotted wilt tospovirus in relation to thrips populations in tobacco crops in northern Greece: E.K. Chatzivassiliou, I. Zintzaras, G. Jenser and N.I. Katis.

P-S4-10- Spread of beet mosaic potyvirus after inoculating the crop at different dates.: A.N. Dusi, D. Peters and W. van der Werf.

11:00 – 11:30 Coffee Break

11:30 – 13:30 ***SESSION 5: Epidemiology of arthropod-borne viruses***

Chairs: F. Morales, Y. Robert and M. Nelson

ORAL PRESENTATIONS:

11:30 – 11:45 Tomato spotted wilt tospovirus and vectors in Portugal: **A-M. Pereira**, I. Cortés, A. Aires, A. Sottomayor, L. Moura, D. Louro, G. Nolasco, L. Almeida, N. Ramos and E. Fernandes.

11:45 – 12:00 Incidence and epidemiology of citrus tristeza virus in the Valencian Community of Spain: **M. Cambra**, M.T. Gorris, M.P. Román, C. Marroquín, A. López, T.R. Gottwald, M.C. Martínez and A. Hermoso de Mendoza.

12:00 – 12:15 Spread of rice yellow mottle sobemovirus as deduced from field observations and experimental results: **D. Peters**, S. Sarra, P. Oevering, Y. Idoe and D. Guindo.

12:15 – 12:30 Epidemiology of the carrot motley dwarf virus complex in parsley: **P. Vercruysse**, F. Meert, P. Bleyaert, L. Tirry and M. Hofte.

12:30 – 12:45 Epidemiology and control of wheat dwarf: **M. Lindblad**, M. Sandgren and R. Sigvald.

12:45 – 13:00 Characterization of iris yellow spot tospovirus and its transmission by thrips: **A. Gera**, A. Kritzman and B. Raccah.

13:00 – 13:15 Epidemiology of potyviruses infecting maize in the Mediterranean basin Spain: **M.A. Achon** and M. Sobrepere.

13:15 – 13:30 Epidemiology and management of tomato spotted wilt tospovirus: **H.R. Pappu**, J.W. Todd, D.G. Riley, A.S. Csinos, P.F. Bertrand, R.J. McPherson and A.K. Culbreath.

POSTER PRESENTATIONS:

P-S5-1- Epidemological studies of virus diseases of faba bean in Egypt: E.K. Allam, N.M. A. Aref and M.L.M. Salama.

P-S5-2- Broad bean wilt fabavirus infecting medicinal and aromatic plants in Italy: C. Rubies-Autonell and M.G. Bellardi.

P-S5-3- Virus diseases of lavender plantations in Crimea: N.N. Senchugova.

P-S5-4- Rice yellow mottle virus disease in two rice cropping systems of Tanzania: F.H. Ali, F.M. Kimmins and D. Overfield.

P-S5-5- Prevalence of virus diseases and the incidence of yam mosaic virus in yam (*Dioscorea* spp.) fields from Bassar and Sotouboua Prefectures of Togo: M.Y.D. Gumedzoe, F. Feteke, Y. Sanwogou and G. Thottappilly.

P-S5-6- Some observations on maize streak disease as a constraint of maize production in west Kenya: M.E. Omunyin.

P-S5-7- Present status of viruses of vegetable crops in southern Italy: C. Vovlas, O. Potere, M.M. Finetti Sialer, and D. Gallitelli.

P-S5-8- Ornamentals, weeds and thrips fauna associated with tospoviruses in Greece and southern Italy: E. K. Chatzivassiliou, I. Livieratos, G. Jenser, C. Vovlas and N.I. Katis.

P-S5-9- Occurrence of African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) in some cassava cultivars (*Manihot esculenta*) and weeds in Togo: M.Y.D. Gumedzoe, D.K. Adjata, B.J.M. Verduin and G. Thottappilly.

P-S5-10- Epidemiology of raspberry bushy dwarf virus in raspberry and blackberry in Czech Republic: J. Spak and D. Kubelkova.

P-S5-11- Epidemiology of grapevine leafroll in central Po Valley, northern Italy: G. Belli, A. Fortusini, G. Scattini.

P-S5-12- Epidemiology and monitoring of some plant viruses in Ukraine: V.P. Polischuk, I.G. Budzanivskaya and A.L. Boyko.

P-S5-13- A cereal disease caused by mite-transmitted viruses: R. Salomon.

P-S5-14- Identification and detection of viruses of *Zantedeschia*: A.F.L. M. Derks, M.E.C. Lemmers, P.J. van Leeuwen and S.A. Langeveld.

P-S5-15- Characterization of an unusual poty-like virus infecting tomatoes in the central Sudan: El Shafie, K. Gebre-Selassie, P. Gognalons, G.A. Dafalla, Y.F. Mohamed, B. Delecolle and G. Marchoux.

P-S5-16- Epidemiology of a maize virus disease transmitted by a planthopper in the Mediterranean area: P.A. Signoret.

P-S5-17- Forecasting potato virus Y using suction traps: R. Sigvald and M. Lindblad.

P-S5-18- Comparison of aphid sampling methods in citrus. A. Hermoso de Mendoza, E. Pérez, E.A. Carbonell and V. Real.

P-S5-19- Epidemiology of zucchini yellow mosaic virus in The Netherlands: J. Th. J. Verhoeven and J.W. Roenhorst.

P-S5-20- Transmission of zucchini yellow mosaic potyvirus by different aphid species not colonizing cucurbits: N.I. Katis, J.A. Tsitsipis, D.P. Lykouressis, A. Papapanayotou, G.M. Kokinis and I.N. Manoussopoulos.

P-S5-21- Transmission of rice yellow mottle virus by the chrysomelid beetle, *Trichispa sericea*: F. M. Kimmins, S.V. Green, R.A. Cheke, P.C. Stevenson and F.H. Ali.

P-S5-22- Rearing of *Cerotoma arcuata* Oliv. (Coleoptera; Chrysomelidae) and its efficiency in transmitting an isolate of cowpea severe mosaic virus: F.J.S. Salas; M.M. Barradas and J.R.P. Parra.

P-S5-23- Incidence and dispersal of bean common mosaic and bean common necrotic mosaic potyviruses in bean fields of Castilla plains of Spain: G. Cifuentes, S. Castro and J. Romero

P-S5-24- Epidemiology of chickpea chlorotic dwarf virus in faba bean and chickpea in Sudan: M.M. Hassan, G.A. Dafalla, J. Vetten and B. Gronenborn.

P-S5-25- Incidence and spread of lily symptomless and lily mottle viruses in lilies in The Netherlands: C.J. Asjes and G.J. Blom-Bamhoom.

P-S5-26- Incidence and spread of thrips -borne tomato spotted wilt virus in dahlia in The Netherlands: C.J. Asjes and G.J. Blom-Bamhoom.

P-S5-27- Studies on yield and virus infection of a garlic collection: I. Camele, V. Candido, G.L. Rana and V. Miccolis.

P-S5-28- Survival of tomato spotted wilt virus under continental climatic conditions: R. Gáborjányi, G. Jenser and M. Graselli.

P-S5-29- Longevity of hibiscus latent ringspot nepovirus in seed of kenaf: C. Rubies-Autonell.

P-S5-30- The recent decrease of virus yellows of sugar beet in middle Germany: G. Proeseler, E. Schliephake, P. Rücker and H. Hartleb.

P-S5-31- Epidemic outbreak of tomato spotted wilt virus in potato fields in the central region of Portugal: C. Serra.

P-S5-32- Vector dependency by viruses: insight into virus-mediated changes in host plants that affect vector performance: S.J. Castle.

P-S5-33- Cucumber mosaic virus in alternative pulse and annual pasture legumes: susceptibility and seed transmission: L.J. Latham, S.J. McKirdy and R.A.C. Jones.

P-S5-34- Quantifying the relationship between seed size in narrow-leafed lupin and incidence of cucumber mosaic virus: F.W. Nutter, Jr., E. Alberts and D. Gratz.

P-S5-35- Surveys of viruses affecting pepper (*Capsicum annuum L.*) in Tunisia: M. Mnari Hattab, K. Ezzaier, K. Gebre Selassie, G. Marchoux and P. Gognalon.

AFTERNOON

13:30 – 15:30 Lunch and Poster Inspection

15:30 – 17:30 ***SESSION 6: Management and control strategies***

Chairs: F. Ponz, H. Lecoq and S. Cohen

ORAL PRESENTATIONS:

15:30 – 15:45 Field expression, variability, and virus strain specificity of replicase gene-mediated resistance to potato leafroll virus: **P.E. Thomas**, J.C. Zalewski, G.L. Reed, E.C. Lawson and W.K. Kaniewski.

15:45 – 16:00 Strain structure of potato virus Y (PVY) and its implications in the specificities of PVY resistance in pepper: **F. Ponz**.

16:00 – 16:15 Inmunity of the transgenic C-5 plum to aphid-inoculation: **M. Ravelonandro**, T. Malinowski, B. Zawadzka and R. Scorza.

16:15 – 16:30 Characterization of attenuated engineered viral cDNA of zucchini yellow mosaic virus for cross protection in cucurbits: **A. Gal-On**, Y. Wang, R. Chemo and G. Yarden.

16:30 – 16:45 Resistance to the causal agent of blackcurrant reversion disease and to its gall mite vector provides effective control of these organism in the field, but for how long?: **A.T. Jones**, R. Brennan, W. McGavin, L. Pullikanti, B. Fenton and A. Lemmetty.

16:45 – 17:00 Optical barriers: an innovate IPM tool for the control of insects pests and virus diseases in protected crops: **Y. Antignus**, M. Lapidot and S. Cohen.

17:15 – 17:15 Development of integrated disease management strategies for two non-persistenly aphid-transmitted viruses infecting lupin crops: **R.A.C. Jones**.

17:15- 17:30 IPM and plant viruses: trying to fit a square peg into a round hole?: **M.E. Irwin** and G.E: Kampmeier.

POSTER PRESENTATIONS:

P-S6-1- Capacity of an all-round protecting agent in an integrated plant management strategy: A.E. Wissing, R.A. Sikora and S.N. Irving.

P-S6-2- Selection of resistance-breaking strains of tomato spotted wilt tospovirus: L.J. Latham and R.A.C. Jones.

P-S6-3- Characterization of soybean mosaic virus coat protein-mediated resistance in transgenic soybeans: F.W. Nutter, Jr., J.H. Hill, X. Wang and A.L. Eggenberger.

P-S6-4- Studies on the translocation of tomato spotted wilt tospovirus in potatoes: C.R. Wilson.

P-S6-5- Evaluation of reflective mulching in combination with insecticide sprays for control of aphid-borne viruses of flower bulbs: C.R Wilson.

P-S6-6- Can pyrethroid and imidacloprid insecticides be used to control spread of cucumber mosaic virus in Lupin?: D.J. Thackray , R.A.C. Jones , A.M. Bwye and B.A. Coutts.

P-S6-7- Studies on the resistance of a *Cucumis melo* accesion to watermelon mosaic virus-2: A. Yassein, R. Camero, M.L. Gómez-Guillamón and E. Moriones

P-S6-8- Evaluation of fipronil for controlling tomato spotted wilt tospovirus transmission: E.I. Garzo, B. Díaz and A. Fereres.

P-S6-9- Barrier crops to control non-persistently transmitted viruses of pepper crops in Spain: Y. Rieche and A. Fereres.

P-S6-10- Evaluation of elite breeding lines having resistance to rice tungro viruses: R.C. Cabunagan, E. Angeles, G.S. Khush, E.R. Tiongco and T.C.B.Chancellor.

P-S6-11- Evaluation of transgenic winter wheat for resistance to barley yellow dwarf and wheat streak mosaic viruses: N.A. Bosque-Pérez, P.H. Berger, J. Rohozinski, P.L. McCarthy, J. Hansen, J.D. Ophus, P.J. Shiel and R.S. Zemetra.

P-S6-12- Eradication of tobacco rattle virus from soils by growing weed-free alfalfa: P.E. Thomas, H. Mojtahedi, J.M. Crosslin and G.S. Santo.

P-S6-13- Viral diseases of grain crops transmitted by arthropods: L.T. Mishchenko, A.M. Silayeva, G.V. Reshetnick and I. M. Plastun.

P-S6-14- Influence of aphid population dynamics on potato virus Y dissemination in tomato crops: J.R. Estévez, A. Carnero, A. Espino, E. Kiss and I. Kajati.

17:30 – 18:00 Coffee Break

18:00 – 19:00 Discussion of Sessions 4, 5 & 6 and Posters

19:00 – 19:30 ***General discussion and future prospects of plant virus epidemiology***

Chairs: R. Jones, M. Thresh, M. Irwin, F. Nutter and A. Fereres

21:00 Conference Dinner

FRIDAY, APRIL 16

MINI SYMPOSIUM: LOCAL PROBLEMS RELATED TO INSECT-TRANSMITTED VIRUS DISEASES IN VEGETABLES: EPIDEMIOLOGY AND APPLICATION OF CONTROL STRATEGIES

9:30 - 10:00 Introduction

Whitefly-transmitted viruses

10:00 – 10:30 Virus transmitted by whitefly: tomato yellow leaf curl virus (TYLCV). Control strategies: M.I. Franco, A. Castillo-Garriga, I. Donoso and **E. Rodriguez Bejarano**.

10:30 – 11:00 Managing whiteflies (*Bemisia tabaci)*, strain B in an agricultural system : **N. Toscano**, N. Prabhaker, S.J. Castle and T. Henneberry.

11:00 - 11:30 Coffee Break

Other insect-transmitted viruses

11:30 - 12:00 Thrips-transmitted viruses: tomato spotted wilt virus. Control strategies: **A. Lacasa Plasencia** and J.A. Sánchez Sánchez.

12:00 - 12:30 Cultural control of insect-transmitted viruses: **Y. Antignus**

12:30 - 13:00 Resistance to aphid-borne viruses in vegetable crops: **H. Lecoq** and M. Pitrat

13:00 - 13:30 General Discussion

OPENING ADDRESS

THE PAST 100 YEARS IN PLANT VIRUS EPIDEMIOLOGY AND THE CONTINUING ECOLOGICAL TRADITION. THE ROLE OF THE ISPP EPIDEMIOLOGY COMMITTEE.

J M Thresh

Chairman of the Plant Virus Epidemiology Committee

The Plant Virus Epidemiology Committee of the International Society of Plant Pathology (ISPP) was established at the International Congress of Plant Pathology in Munich, Germany, in 1978. The overall objective was to promote studies on plant virus epidemiology and control and to foster liaison and collaboration between virologists and those concerned with animal and fungal vectors. One of the principal incentives for forming the committee was concern at the time that the biology of viruses was being neglected or even overwhelmed by the advancing tide of biochemistry.

The main activity of the Committee has been to initiate a series of International Symposia arranged by local organizers in different countries. Symposia have been held in Oxford, UK (1981), Corowa, Australia (1983), Orlando, USA (1986), Montpellier, France (1989), Bari, Italy (1992), Jerusalem, Israel (1995) and now Aguadulce, Spain.

During the current symposium there will be an open 'business' meeting to discuss the future activities of the Group and to nominate or elect a new committee and chairperson. The Committee reports to ISPP Council which provides support but, so far, little financial backing. A requirement of ISPP Council is that the Committee includes representatives from each geographic region.

The Aguadulce symposium is being held in the final year of the 20th century and one year after the 1998 commemoration of 100 years of Plant Virology. This provides an incentive to assess the current status of plant virus epidemiology and some of the main achievements of the last 100 years. It is also appropriate to consider the range of material being presented in 1999 in relation to the main themes of previous symposia and to earlier developments in plant virus epidemiology.

Some of the topics to be considered will be the different means of virus dispersal and the diverse range of animal and fungal vectors involved. This diversity and the need for a wide-ranging ecological approach were appreciated at an early stage from studies on sugar beet curly top and other viruses in the United States. An ecological tradition was established and can still be discerned, although at times it has been tenuous and close to extinction.

This is no longer the situation, as evident from the abstracts submitted and the large number and wide range of participants who have registered for the latest symposium. Clearly, plant virus epidemiology thrives and much use is being made of the new techniques and innovations becoming available. It is also apparent that solutions are being found to the new and continuing problems that affect a wide range of crops. Nevertheless, problems remain, especially in the tropics and sub-tropics, as evident from the many presentations on whitefly-borne viruses in parts of the Americas, Africa and Asia, where it is particularly important to increase food production in the face of burgeoning human populations. Other issues requiring attention are the need to decrease the use of pesticides and to consider the ecological implications of genetically modified organisms and the most effective ways in which they are deployed. This emphasises the continuing role of epidemiology and the challenges that remain as we move towards the next century.

SESSION 1

TRANSMISSION MECHANISMS OF PLANT VIRUSES

ORAL PRESENTATIONS

ELECTRICAL ANALYSIS OF NON-CIRCULATIVE VIRUS TRANSMISSION

A. Fereres[(1)] and J. L. Collar[(2)]

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[(2)] Cyanamid Iberica S.A., Crop Protection Division, Madrid, Spain.

In recent years there has been a considerable effort to elucidate and understand the transmission mechanisms and relationships between plant viruses and their insect vectors. One technique that has become essential in studies concerning the transmission mechanisms of plant viruses and the existing interactions within the insect-virus-host plant pathosystyem is the electrical monitoring of insect probing and feeding behavior.

Non-persistent viruses, are transmitted almost exclusively by aphids. This suggests the existence of one or more important morphological, physiological or behavioural traits that separates aphids from other insect vectors of plant viruses. Our laboratory has concentrated on the electrical analysis of probing and feeding activities of aphid vectors associated to the transmission of non-persistent viruses. We found that acquisition of non-persistent viruses (CMV and PVY) occurs primarily during the last sub-phase (II-3) of intracellular stylet punctures (= potential drops), whereas inoculation is achieved during the first sub-phase (II-1). Also, some aphid species display long potential drops (pd-L), characterized by a specific sub-pattern structure with a sub-phase II-3 containing several archlets (3 or more at about 2 Hz frequency). This type of pd-L occurs most frequently during the first brief superficial probes and its duration has been positively correlated to non-persistent virus transmission of CMV by *A. gossypii*. Interestingly, pd-L waveform has never been recorded in other groups of insect vectors studied so far, such as whiteflies, leafhoppers or mealybugs.

In experiments carried out with *M. persicae* subjected to a 5min acquistion access period on a PVY-infected pepper plant we observed that there was a negative correlation between the time elapsed from the last intracellular puncture to the end of the probe and the ability to transmit PVY. This result indicates that virus inoculability begins to decrease immediately after virus acquistion and it is very likely to happen as a result of aphid activities during stylet pathway (e.g. salivation).

We have also found that *M. persicae* subjected to a 1h starvation period were able to produce a longer sub-phase II-3 during their first intracellular puncture than unstarved aphids. This difference may explain why starvation increases the transmission rate of non-persistent viruses. A similar decrease in the duration of sub-phase II3 (and in the number of II-3 pulses) has also been observed for *M. persicae* and *A. gossypii* when previously fasted aphids were allowed to produce consecutive potential drops within the same probe.

Finally, we found that the most efficient vectors of PVY (*M. persicae* and *A. gossypii*) produce a very long II-3 sub-phase and several pulses during their first potential drop produced

within the first probe. Conversely, poor vectors of the virus (*R. padi* and *S. avenae*) produced potential drops with a very short II3 sub-phase and in many cases absence of pulses. This differential behavior may explain at least partially differences in vector transmission efficiency.

POTYVIRUS TRANSMISSION BY APHIDS: VIRION-HELPER COMPONENT-STYLET INTERACTIONS

T.P. Pirone and R.Y. Wang

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Potyvirus transmission involves the virion capsid protein (CP) as well as a non-structural protein "helper component" (HC). HC functions by acting as a "bridge", interacting with both virions and the food canal of the stylets to allow retention of virions at a site from which they can be inoculated. Ultrastructural and autoradiographic studies have shown that transmission is associated with retention of virions in the distal portion of the food canal formed by the maxillary stylets.

Changes in an asp-ala-gly "DAG" sequence near the surface-exposed N-terminus of the CP reduce or abolish transmissibility. Aphids fed on mixtures of virions and functional HC retain in their stylets virions with a DAG motif, but not those with a DAE motif. Dot-blot binding and protein-blotting overlay assays reveal a correlation between transmissibility of CP mutants and the ability of the mutants to bind HC. Mutations in two highly conserved HC motifs result in loss of HC activity. A thr to ala mutation in a conserved PTK motif results in loss of virion- binding ability of HC, whereas a lys to glu mutation in the KITC motif results in inability of HC to be retained in the stylets, but does not affect binding of HC to virions.

Two other types of experiments also demonstrate the importance to transmission of HC-mediated virion retention in the stylets. Aphids that probe through films of mineral oil either prior to or following acquisition probes seldom transmit tobacco etch virus (TEV) and seldom retain virions in the stylets. Aphids starved prior to acquisition access more often retain virions in the stylets and transmit with greater efficiency than non-fasted aphids.

The effect of HC on the specificity and efficiency of transmission of TEV and turnip mosaic (TuMV) potyviruses was studied with four aphid species. HC fully functional in *Myzus persicae* was not functional in two other species. This suggests that constituent(s) of or in the food canal of particular aphid species differ in the ability to interact with specific Hcs. This leads to differences in retention and subsequently transmission of specific potyviruses. The results of ongoing studies with other HCs and other aphid species will be discussed.

MEDIATED TRANSMISSION OF ZUCCHINI YELLOW MOSAIC VIRUS AND OTHER POTYVIRUSES USING HELPERS PURIFIED BY THE NI^{2+}-NTA RESIN

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The conventional method to obtain the helper component (HC) protein was to separate it from virions by ultra-centrifugation. Attempts to further purify or concentrate the zucchini yellow mosaic virus (ZYMV) HC were mostly not successful. Therefore, an attempt was made to obtain a purer preparation of ZYMV-HC using the Ni^{2+}-NTA resin. Histidine-tagged and non-tagged ZYMV-HC was found to bind to Ni^{2+}-NTA resin. Purification of HC by this method was fast (2-3 h) and efficient, yielding large amount of predominantly HC. High titers of HC protein were also obtained from plants infected with three other potyviruses [watermelon mosaic virus II (WMVII), papaya ringspot virus (PRSV) and turnip mosaic virus (TuMV)]. In contrast, the HCs of potato virus Y (PVY) and tobacco vein mottling virus (TVMV) did not bind to the Ni^{2+}-NTA resin. All the HCs that were purified by the Ni^{2+}-NTA resin were bound *in vitro* to membrane-blotted virions. The ability of Ni^{2+}-NTA-HC to assist transmission was compared to that obtained by centrifugation. The specific activity (rates of transmission as a function of amount) of HC purified by the Ni^{2+}-NTA method were lower than those of HC obtained by centrifugation. Helpers purified by the two methods were compared for their ability to assist homologous and heterologous transmission of potyviruses. The significance of these findings and the potential of this method for studying HC of different potyviruses is discussed.

MOLECULAR MECHANISMS OF CAULIFLOWER MOSAIC VIRUS APHID TRANSMISSION: UNRAVELLING INCREASING COMPLEXITY

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Cauliflower mosaic virus (CaMV) is transmitted by aphids in a non-ciruclative manner. Efficient transmission is dependent on the presence of a non structural, virus-encoded protein, designated aphid transmission factor (ATF). The ATF is the product of the virus gene II and has a molecular weight of 18 kDa. Though a large number of properties have been described for the ATF, the complete picture of its mode of action remains unclear. The CaMV gene II product accumulates in infected plant cells as a highly organized “paracrystalline structure” which was proposed to be a reservoir wherefrom active ATF can be solubilized. The C-terminus of the ATF mediates a strong and specific interaction with the virions and this interaction was demonstrated to be necessary for successful aphid transmission. In addition the ATF can form a highly stable complex with cellular microtubules in plant, insect and mammalian cells but, unfortunately, the role of this complex in the process of CaMV aphid transmission is not understood.

Recent data further illustrates what seems to be an evergrowing complexity in the molecular mechanisms of the CaMV aphid-transmission.

1 - The ATF-virion binding was found to be mediated by an additional component, being the product of the CaMV gene III (PIII). PIII was demonstrated to interact, via two distinct domains, with both the ATF and the purified virions, thus putatively bridging between the two.

2 - The ATF-PIII-virion complex is to be assembled according to a precise sequence in order to be aphid-transmissible. An ATF-PIII complex does not assist the aphid transmission of purified virions whereas the ATF alone can assist transmission of a preformed PIII-virion complex. A model presenting PIII as a factor regulating the activity of the ATF will be discussed.

3 - Finally, we will present preliminary evidences suggesting that the CaMV ATF requires at least one additional, yet unidentified, function for mediating virus transmission by aphids.

GEMINIVIRUSES: INTRACELLULAR PATHWAY AND TRANSPORT SITES IN THEIR INSECT VECTORS.

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Specific species of leafhoppers, whiteflies and a treehopper are all vectors of geminiviruses. Vectors of one virus are usually restricted to species in one genus; often only a single species. Geminiviruses are single-stranded DNA viruses which are transmitted in a circulative and persistent manner. The transmission process is partitioned into uptake, acquisition, retention and inoculation and each stage is influenced by a number of interactions. *Cicadulina* leafhoppers transmitting maize streak virus (MSV) have a barrier to acquisition controlled by a single dominant sex-linked locus. *C. mbila* populations have been bred which exhibit these phenotypes. In contrast, all geminiviruses in the *Begomovirus* genus are transmitted by *Bemisia tabaci*, the tobacco whitefly, but some of these viruses can be acquired non-specifically by aphids and other genera of whiteflies, but not transmitted. In both leafhoppers and whiteflies the filter chamber appears to be the site of transmembrane passage between gut lumen and haemolymph. In *C. mbila*, the most efficient vector of maize streak virus, the virus is endocytosed into receptosomes and transported to the basal membrane; once in the haemolymph the virus circulates to the salivary glands where the particles are absorbed into specific cells, ultimately for inoculation in the saliva. This insect pathway has been elucidated using immunogold labelling for examination by electron microscopy of virus particles in vector and non-vector insects and insect non-transmissible isolates , as well as with different combinations of virus and vector species.

COAT PROTEIN GENE REPLACEMENT RESULTS IN WHITEFLY-TRANSMISSION OF AN INSECT NON-TRANSMISSIBLE GEMINIVIRUS ISOLATE

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Gemiviruses are transmitted by whiteflies, leafhoppers or treehopers. The whitefly species *Bemisia tabaci* (Gennadius) is the most efficient vector of members of the genus *Begomovirus* (previously known as subgroup III). The majority of members of this genus have bipartite genomes (DNAs A and B). Abutilon mosaic virus (AbMV), a bipartite geminivirus, was acquired but not transmitted by *B. tabaci*. In contrast, Sida golden mosaic virus (SiGMV-Co), a closely related geminivirus, was acquired and transmitted efficiently by *B. tabaci* to various host plants. The coat protein of AbMV was replaced with that of SiGMV-Co. The resulting chimeric AbMV was acquired and transmitted by *B. tabaci*, indicating that determinants for transmission by *B. tabaci* are located on the coat protein.

SEX-MEDIATED TRANSMISSION AND PROPAGATION OF TOMATO YELLOW LEAF CURL GEMINIVIRUS BY WHITEFLIES

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The possibility that tomato yellow leaf curl virus (TYLCV) is transmitted among whiteflies during sexual intercourse was investigated. Males that were fed for 24 h on infected plants were mixed with an equal number of non-viruliferous females and allowed to feed on artificial diet through membranes for various periods of time (4 h to 48 h). The females were then tested individually fo the presence of TYLCV by PCR. The percentage of females that acquired the virus increased with time, reaching up to 50% of the individuals after 48 h. In the reciprocal experiment TYLCV was transmitted by viruliferous females to males. TYLCV was not transmitted to insects of the same sex. The insects that have acquired TYLCV from their sexual partners were able to infect tomato plants with an efficiency of up to 30% using one insect per plant.

The effect of sexual transmission of TYLCV on the spread of the virus in a whitefly population was tested. Three pairs of viruliferous insects were mixed with 200 non-viruliferous individuals picked from the insect colony (about 1/3 males, 2/3 females) and fed on artificial diet. The entire population was analyzed during repeated independent trials. The results showed that the number of viruliferous insects increased with time reaching more than 50% of the population, males as well as females, 8 days after the experiment started.

The contribution of sexual transmission of TYLCV in insect populations to infection of tomato was studied by mixing three pairs of viruliferous insects with 200 non-viruliferous insects. After 48 h on artificial diet, groups of 3 insects were picked randomly and were caged each with a tomato test plant. Observation of symptoms and Southern blot analyses indicated that the number of infected plants was about 60% higher than the number of plants that should have been infected if sexual transmission of the virus did not occur.

Considering the deleterious effects of TYLCV on its whitefly vector, transovarial transmission and sex-mediated spread of this virus in insect populations, we consider TYLCV to be primarily a sex-transmitted pathogen of whiteflies.

INFECTION OF TRANSMITTING AND NON-TRANSMITTING THRIPS BY TOMATO SPOTTED WILT VIRUS

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Tomato spotted wilt virus is transmitted biologically by some thrips species. Only three stages, the two larval stages and the adults, feed on plants and are thus involved in the transmission of this virus. The larvae acquire the virus, whereas the adults cannot do so. After acquisition, larvae in the end of the second stage and adults can transmit the virus. When young first instar *Frankliniella occidentalis* larvae acquire the virus, 70 to 80 % of the second stage larvae can transmit after replication of the virus during a latent period which is temperature-dependent. Analysis of the transmission competence and infection of the thrips showed that *c.* 50 % of the thrips which have ingested virus can transmit it. The virus can be detected in these thrips by ELISA, but also in 20-25 % of the non-transmitters. The transmitting thrips carry usually a higher virus load than the non-transmitters. No virus is detected in the rest of the non-transmitting thrips.

Analysis of the midguts of *F. occidentalis* larvae reveals that all larvae are infected two days after an acquisition access period of 16 hours. The first infections were observed in the epithelium of the first midgut region and spread then to the visceral muscle tissues of the first region and subsequently to the middle and posterior region. In the adult stage, infections were also discerned in the foregut. After becoming adult, the virus could no longer be observed in the midgut epithelium. The level of midgut infection varied considerably between the different individual thrips. The virus could also be detected in the salivary glands. The glands of those which transmit were thoroughly infected. The infection in glands is restricted to a small area in non-transmitting thrips or could not be detected. The various levels of midgut and salivary gland infection after pupation explain the ELISA results.

The results presented showed that the infection of larvae does not necessary result in virus transmission. This conclusion was supported by an analysis of the transmission of a TSWV isolate with a defective interference RNA. The midguts of 23 % of the larvae were infected 96 hours after acquisition. After becoming adult, none of the larvae could transmit and only traces of the infection could be observed in the midgut muscle cells.

The drastic renewal of the midgut during pupation results in an almost complete elimination of the virus from the midgut. As the thrips is able to transmit directly after pupation, we conclude that the virus has to reach the salivary glands before pupation to become infectious.

POSTER PRESENTATIONS

PLUM POX VIRUS HELPER COMPONENT: MOLECULAR INTERACTIONS WITH COAT PROTEIN AND SYNERGY WITH POTATO VIRUS X

S. Yang and M. Ravelonandro

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Helper component (HC-Pro) of potyvirus is a virus encoded non-structural and multifunctional protein. For aphid transmission, it is believed that HC-Pro acts as a bifunctional molecule of which one domain binds to the virion's coat protein (CP) and the other one to aphid stylet receptors. Some motifs involved in such an interaction have been proposed for both HC-Pro and CP proteins. In order to test *in vitro* interactions between the HC-Pro and the CP of plum pox virus, we have introduced some mutations into these motifs, for instance: "KITC"→"EITC" and "PTK"→"PTE" for the HC-Pro protein and "DAG"→"ΔDAG" for the CP protein. The mutated HC-Pro genes were cloned into the PVX vector and expressed transiently in *Nicotiana benthamiana*, However, the wild-type and the mutated CP proteins were cloned into an *E. coli* expression vector and produced by *E. coli* expression system.

The interaction tests using the "protein blotting-overlay technique" demonstrated a direct interaction *in vitro* between the HC-Pro and CP of PPV. The two mutations in "K(E)ITC" and "PTK(E)" motifs have no evident effects on this interaction. A truncated HC-Pro protein having its amino acid sequence located between the KITC and PTK motifs showed also its interaction with wild-type CP protein. Further analyses with other constructions are being done.

The biolistic inoculation of *N. benthamiana* or *N. clevelandii* with recombinant PVX carrying wild-type HC-Pro gene led to stem and leaf necrosis, and finally to the death of the diseased plants. These symptoms are much more severe in comparison with those of wild-type PVX in these plants. This observation suggests that the HC-Pro protein would be the only viral product responsible for the observed synergy between PVX/potyvirus in the symptom expression. However, the introduction of the two mutations into HC-Pro gene led to the lost of its capacity in inducing the synergistic effects when it is expressed transiently *in vivo* by the PVX vector. Observations concerning two of these aspects will be discussed.

STUDYING THE GENETICS OF VIRUS TRANSMISSION: DEVELOPMENT OF GENETIC SYSTEMS FOR LEAFHOPPERS

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Leafhoppers (family Cicadellidae) are amongst the most important virus vector groups, particularly in tropical and subtropical regions. Despite the economic damage that they cause however, little is known about their genetics and the genetic basis of virus transmission. Our studies have focused on two leafhopper species, the main vector of maize streak virus (MSV) in Africa *Cicadulina mbila* and the green leafhopper of rice *Nephotettix virescens*, the main vector of rice tungro disease (RTD) in South East Asia. These two systems represent two of the main modes of virus transmission by leafhoppers: circulative for MSV and semi-persistent for RTD.

We have been able to confirm and follow up the experiments first carried out by H.H. Storey in the 1920s, which identify a single sex-linked locus that controls the ability of *C. mbila* to transmit MSV. A rapid, PCR-based method was developed for detecting virus circulation, and thus reliably scoring different phenotypes. The effects of different genotypes of the locus, that we named storey (sto) on the phenotype of the insect and virus circulation will also be discussed.

In *N. virescens*, we have studied a number of colour mutants and established a system of visible markers to facilitate genetic studies. The problems and current progress with the system will be discussed.

APHID TRANSMISSION AND LONG-TERM ABSENCE OF SWEETPOTATO FEATHERY MOTTLE VIRUS IN SWEETPOTATO

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Sweetpotato virus disease (SPVD) is a complex disease resulting from infection by the aphid-borne sweetpotato feathery mottle virus (SPFMV) and the whitefly-borne sweetpotato chlorotic stunt virus (SPCSV). Aphid transmission and retention of SPFMV in sweetpotato were tested in a screenhouse during 1996 and 1997. Two sweetpotato cultivars, Tanzania and New Kawogo; moderately resistant and resistant to SPVD, respectively, were used. SPFMV alone had only a low mean virus titre (0.22; healthy control = 0.10) in both sweetpotato cultivars, became increasingly difficult to detect in plants of these cultivars and was seldom acquired by aphids. However, this was not apparent in plants also infected with SPCSV. These SPVD-affected plants then had a high mean SPFMV titre (1.84; healthy control = 0.10), appeared unable to eliminate SPFMV and provided good sources for aphids to acquire.

VECTORIAL VARIABILITY AMONG POPULATION OF *FRANKLINIELLA OCCIDENTALIS* POPULATIONS TRANSMITTING TOMATO SPOTTED WILT VIRUS AND THE PATTERN OF VIRUS SPREAD IN LETTUCE FIELDS IN ISRAEL

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Five thrips populations, three from Bet Dagan (BD-L, BD-P and BD-Man) and two from the Arava (AR-S and AR-P) were compared for their ability to transmit several isolates of tomato spotted wilt virus (TSWV). Thrips larvae were placed on infected *Datura stramonium* for acquisition access feeding. Then, after the completion of their developmental cycle the insects were placed on *Petunia* or *Emilia* leaf discs for a subsequent inoculation access feeding. Virus presence was ascertained both visually and by ELISA. The most efficient thrips population was BD-Man, transmitting a TSWV from *Pittosporum tobira* at 83%, from *Anemone coronaria* at 45% *and* from tomato at 89%. The two other thrips populations from Bet Dagan (BD-1, BD-P) transmitted the TSWV isolates from pepper and *Hippeastrum* at 26 and 10% efficiency, respectively. The AR-P population transmitted the same isolates at 14 and 10%, respectively, while the AR-S failed to transmit either TSWV isolate. These results provide evidence for intra-specific variation in vector competence.

Natural spread of TSWV was estimated by periodical monitoring of infection in commercial lettuce plots. Spread seems to reflect the magnitude of the thrips population. The incidence of thrips was determined by exposure of colored sticky traps in experimental plots. The spatial distribution of the infection suggested a clustered pattern. The reasons for the overall low rate of infection (5%) in the field are discussed.

TRANSMISSION OF TOMATO SPOTTED WILT TOSPOVIRUS BY *THRIPS TABACI* POPULATIONS AND DETERMINATION OF THE MEDIAN LATENT PERIOD

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The transmission efficiency of TSWV by *Thrips tabaci* populations collected on leek and tobacco was studied using leaf discs from *P. hybrida, D. stramonium* or *N. tabacum* cv Basmas. Adult thrips of arrhenotokous populations of *T. tabaci* collected from infected tobacco in the field were highly infectious (up to 48%). These thrips were also efficient transmitters when kept on tobacco in the laboratory; transmission rates were 67%. Thrips of an arrhenotokous leek population were poor transmitters; reaching rates of 3%. No transmission was obtained in tests with thelotokous populations from leek.

The latent period of TSWV was studied using a *T. tabaci* population from tobacco. The majority of thrips started to transmit after becoming adult. The median latent period (LP_{50}) values, found for the adult stage of both sexes, decreased with increasing temperatures. The LP_{50} values were 350, 278 and 220 h for males, 404, 310 and 246 h for females, and 377, 300 and 238 h for the total population of thrips when kept during the whole experiment at 20, 24 and 27 ^{o}C, respectively. The transmission rates were also positively correlated with these temperatures. These rates were 57, 56 and 62% for males, 46, 67 and 68% for females, and 54, 63 and 66% for the whole population.

The number of thrips which transmitted TSWV as larvae was too low to calculate LP_{50} with confidence. The percentages of larvae that transmitted TSWV decreased with increasing

temperatures. They were 17, 11 and 7% for males, 23, 5 and 5% for females, 28, 8 and 7% for the whole population at the respective temperatures. These results indicate that the time interval for larvae between becoming infectious and pupation is smaller at higher temperature, hence a smaller chance to transmit.

MINERAL OIL EFFECT ON *MYZUS PERSICAE* PROBING BEHAVIOR AND SUBSEQUENT POTATO VIRUS Y ACQUISITION FROM PEPPER PLANTS

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The probing behavior of *Myzus persicae* Sulzer (Homoptera: Aphididae) on pepper plants treated with a paraffinic oil was studied using an electronic monitor (EPG technique). With this device, extracellular stylet pathway (waveform C) was distinguished, as well as short intracellular punctures (waveform pd). The intracellular phase of these pd waveform comprised three different subphases: II1, II2 and II3, the last of them correlated with non-persistent virus acquisition.

The probing behavior of *M. persicae* individuals was recorded during 5 min of access to the same pepper leaf before and after treatment with paraffinic oil. Aphids showed a much longer pre-probing time and produced fewer probes after oil treatment, although the mean duration of probes was not different. Also, the application of oil resulted in a decrease of the number of brief punctures (pds), although their mean duration remained similar. Interestingly, the first pd was significantly shorter when produced after oil application. This difference could be explained by a decrease in subphase II3 duration and in the number of typical pulses recorded.

In a different set of records, *M. persicae* individuals were recorded during PVY acquisition from an infected pepper plant before and after oil application. Acquisition was artificially interrupted after the first intracellular puncture (pd) occurred. Aphids were inmediately transferred to individual test pepper seedlings, that were checked for virus infection 30 d later using ELISA. Previous observations were confirmed: pre-probing times were longer and pds were shorter after oil application. Again, a decrease in II3 duration was responsible for the shorter pds. PVY transmission efficiency was much higher when the virus source plant was not treated with oil (45% vs. 10%). Behavioral variables from aphids that transmitted PVY were compared with those from non-transmitters. No correlation between aphid behavior during PVY acquisition and subsequent virus transmission could be found. These results suggest that, although some behavioral changes are induced by oil application, non-behavioral factors are mainly responsible for the inhibitory effect of mineral oils on non-persistent virus transmission.

CORRELATION BETWEEN WHITEFLY FEEDING BEHAVIOR AND TOMATO YELLOW LEAF CURL VIRUS TRANSMISSION

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The feeding behavior of the whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) was monitored using the Electrical Penetration Graph (EPG) technique during the transmission of tomato yellow leaf curl geminivirus (TYLCV). The behavior of individual viruliferous whiteflies was recorded on two-leaf stage tomato test plants (*Lycopersicon esculentum* Mill cv. "Riofuego"). A total number of 213 whitefly individuals were recorded on single test plants using inoculation access periods that ranged from 3.5 to 14 hours. Subsequent TYLCV infection in these test plants was checked 3-4 weeks later by symptom observation and confirmed by enzyme-linked inmunosorbent assay (ELISA). Different EPG waveform patterns, previously correlated with behavioral events in aphid studies, were measured: waveform C (intercellular stylet pathway), waveform $E(pd)_1$ (salivation into phloem) and $E(pd)_2$ (ingestion from phloem).

Individual recordings were classified into four categories depending of the waveforms observed: Group 1, with recordings including only waveform C (pathway), associated with a residual 2.4% (2 out of 83) TYLCV transmission efficiency; group 2, recordings showing stylet pathway and a single $E(pd)_1$ waveform, with 7.7% (2 out of 27) transmission efficiency; group 3, recordings showing stylet pathway and a single $E(pd)_1$ + $E(pd)_2$ waveforms, that achieved a 23.4% (11 out of 47) transmission efficiency; and group 4, recordings including pathways followed by several $E(pd)_1$ + $E(pd)_2$ patterns, associated with the highest transmission efficiency (37.5%; 21 out of 56).

A total of 16 different behavioural variables were introduced into a stepwise-backward logistic regression model to determine which ones were most related to TYLCV transmission. Eight variables were selected as being those associated with TYLCV inoculation. Among them, the total duration of $E(pd)_1$ was the most significant variable (P =0.002, positive relationship) associated to transmission.

A strong positive correlation was established between the number of $E(pd)_1$ and TYLCV transmission efficiency (r^2=0.99, P=0.003), as well as between the total duration of $E(pd)_1$ and TYLCV transmission efficiency (r^2=0.89, P=0.056). Furthermore, 1.8 min. was the minimum time threshold obtained for a succesful phloem inoculation access period ($E(pd)_1$). Implications of these results for persistent virus transmission by whiteflies will be discussed.

HELPER COMPONENT MUTATIONS IN TWO NON-CONSERVED RESIDUES ARE ASSOCIATED WITH LACK OF APHID TRANSMISSIBILITY OF A PEPPER POTATO VIRUS Y ISOLATE.

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Potato Virus Y (PVY) is the type member of the genus *Potyvirus* (family *Potyviridae*), the largest group of RNA plant viruses. PVY isolates infecting pepper (*Capsicum annuum* L.) have been classified into three pathotypes (0, 1 and 1-2) based on their ability to overcome certain resistance genes to PVY infection.

Non-persistent aphid transmission of potyvirus requires, in addition to virions, a virus encoded non-structural protein, "helper component" (HC), which is essential for virion retention in the stylets of the aphid. This protein is considered to act as a bridge between the coat protein (CP) of the virus particle and a putative receptor in the aphid mouthparts. A number of potyvirus strains which are non-transmissible by aphids due to alterations in the amino acids sequence of the HC have been reported so far (Pirone & Blanc, 1996; *Annu. Rev. Phytopathol*. 34, 227-247).

The aphid transmission properties of a pepper PVY isolate of pathotype 1-2 has been characterized. PVY 1-2 was not transmitted in plant-to-plant experiments, although purified virus particles were readily transmitted when supplemented with heterologous HC of the transmissible isolate PVY 0 AT through membrane acquisition assays, indicating that its CP was functional in transmission. Analysis of the PVY 1-2 HC gene sequence and comparison with that of PVY 0 AT revealed nineteen nucleotide differences but only two derived in amino acid changes, one of which induced a change of charge. Neither of these two amino acid changes did occur inside the cysteine rich domain nor did they coincide with conserved motifs of the HC protein known to be involved in aphid transmission and present in all potyviruses known so far. However, both changes are located in highly conserved positions among PVY strains.

POTYVIRUS HELPER COMPONENT GENE REPLACEMENT: EFFECTS ON INFECTIVITY AND APHID TRANSMISSIBILITY.

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Plum pox potyvirus (PPV) causes important diseases of stone fruit trees. In nature, potyviruses are aphid-transmitted in a non-persistent manner with at least two virus-encoded proteins involved in the process: the helper component (HC) and the coat protein (CP). Besides aphid transmission, HC is a multifunctional protein which has been shown to be required in several processes involved in viral infection.

It has been reported that HC of some potyviruses can promote the heterologous aphid transmission of some other members of the group. We have analyzed the aphid transmission of a non-transmissible natural Spanish isolate of PPV (PPV 3.3) harboring a deletion of 15 amino acids at the N-terminus of the CP which affects the DAG motif involved in aphid transmission. Also a NAT variant of PPV with the same deletion described for PPV 3.3 obtained by mutagenesis of a full-length cDNA clone of the PPV Rankovik genome was used for transmission experiments. Both purified viruses were readily transmitted by aphids if they were supplemented with purified HC obtained from a transmissible potato Y potyvirus (PVY 0 AT), in membrane acquisition assays. In order to test

further this heterologous system, we have constructed chimeras by exchanging the HC of Rankovik PPV for that of PVY in the full-length PPV Rankovik cDNA clone. Two different constructions were done: one using the HC gene from the transmissible PVY 0-AT and another with a non-aphid transmissible PVY 0-NAT HC gene. PVY 0 AT and PVY 0 NAT HC proteins differ in only one amino-acid change which has been shown to be involved in the lack of PVY 0 NAT HC activity. The chimeric cDNA clones, under the control of the cauliflower mosaic virus 35S promoter, were directly inoculated on *Nicotiana benthamiana* and *N. clevelandii* plants. The effects of the presence of the different PVY HC on infectivity and aphid transmissibility of PPV will be presented.

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SESSION 2

CURRENT APPROACHES TO PLANT VIRUS EPIDEMIOLOGY

ORAL PRESENTATIONS

NON-PERSISTENT AND SEMI-PERSISTENT VIRUS DETECTION IN SINGLE APHIDS BY SQUASH CAPTURE-PCR.

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Squash capture-PCR (SC-PCR), a variant of print capture-PCR technique (Olmos *.et al*., 1996; *Nucleic Acid Research* 24**,** 2192-93), has been applied for virus detection in single aphids. This method allows the immobilization of viral targets on Whatmann 3MM paper by squashing the aphids, their storage at room temperature and release with Triton X-100 prior to amplification by PCR without a preliminary viral nucleic acid extraction. This system of sample preparation coupled with a combined assay of reverse transcription and hemi-nested or nested-PCR or PCR-ELISA, allowed the detection of targets from different isolates of non-persistent viruses (plum pox-PPV and potato virus Y-PVY potyviruses) and the semi-persistent citrus tristeza closterovirus (CTV) in several aphid species regardless of their transmission efficiencies. The aphid species tested were *Myzus persicae*, *Aphis gossypii*, *Hyalopterus pruni* and the non-stone-fruit-tree-colonizing species *Aphis nerii* for PPV; *M. persicae* for PVY and *Toxoptera citricida*, *Aphis gossypii*, *Toxoptera auranti*, *Aphis spiraecola*, *A. nerii* and *H. pruni* (non described as a CTV vector) for CTV.

The method also made possible the simultaneous detection and typing of different aggressive (M) and common PPV isolates and the discrimination between aphid and non-aphid transmissible (NAT) PPV isolates from single aphids. The segregation of different isolates from a mixture by a single aphid was confirmed.

The SC-PCR technique offers a new, simple and useful tool for studying the mechanism of acquisition and location in the vector of both semi- and non-persistent viruses. In addition, the success in amplifying viral targets from pre-squashed aphids captured in nature opens new possibilities for plant virus epidemiological studies.

DETECTION AND DIFFERENTIATION OF STRAINS OF CITRUS TRISTEZA VIRUS BEING TRANSMITTED IN FLORIDA SINCE THE ESTABLISHMENT OF *TOXOPTERA CITRICIDA* (KIRKALDY), THE BROWN CITRUS APHID

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Citrus tristeza virus (CTV) occurs in most citrus producing regions of the world, and it is the most serious viral pathogen of citrus. With the recent establishment of the brown citrus aphid, (BrCA) *Toxoptera citricida*, its most efficient vector in Florida and the Caribbean Basin countries, the impact of CTV in the region is likely to increase. Since CTV is known to occur frequently as a mixture of several strains, the strain complexity also is likely to increase. The undetected minor populations can be important to disease management, especially in nurseries and mild strain cross protection situations. These minor populations may become major populations under changed environmental conditions, or as a result of graft/insect transmissions. Hence, detection and awareness of these minor populations of CTV strains is very important.

Surveys were conducted and citrus samples taken in specific areas both prior to and following the establishment of the BrCA. The method used for strain differentiation was based on minor sequence variations that are conserved in the capsid protein genes (CPG) of different strains of CTV and the ability of our strain specific probes to detect and differentiate them. The CPGs or larger genomic segments were amplified by reverse transcription/PCR, and probed directly, or they were T-A cloned (Promega) and the clones separated and maintained in single bacterial colonies. These clones were then screened with strain-specific probes by using either PCR-amplification of the CPGs, or by direct colony lifts.

Probing of CPGs amplified by PCR from CTV-infected trees has enabled the detection of up to four strains of CTV infecting the same tree. However, when only a single probe reacts with the PCR product, it is not clear whether that tree is infected with that single strain of CTV, or whether minor populations of CTV strains are present below the level of detectability by dot blot hybridization. Cloning the PCR products of CPGs from infected tissue and then screening of several clones by hybridization revealed that frequently there are minor populations of CTV strains present in infected trees. This approach, as confirmed by sequencing, is more sensitive than previous approaches for detecting and differentiating strains of CTV, and it has the added advantage of providing clones of the minor populations for characterization and further elucidation of the diversity of CTV. These methods enabled us to demonstrate the presence and movement of stem pitting strains of CTV previously undetected in Florida, and which now present a significant threat to citrus production in Florida and throughout the Caribbean region, especially with the widespread establishment of the BrCA throughout the region.

ANALYSIS OF THE GEOGRAPHICAL DISTRIBUTION OF COTTON LEAF CURL DISEASE-ASSOCIATED BEGOMOVIRUSES IN PAKISTAN

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Leaf curl disease of cotton in Pakistan is associated with a number of different species of the genus *Begomovirus*. Primers and probes specific for these viruses were developed for their detection by PCR and dot-blot hybridization, respectively. Samples of infected plants were collected from cotton growing districts of the Punjab where the disease is causing major yield losses. Infected samples were also collected from cotton grown in the Sindh, where the disease has only recently arrived, as well as from Balochistan and North Western Frontier Province. Samples were tested for the presence of these viruses to determine their distribution in the field. Infection by Begomoviruses correlated with the presence of disease symptoms in the field. Multiple infection with more than one Begomovirus was detected in most of the samples. However the viruses were also found in single infections with similar symptoms. The role of multiple infection in the evolution of these viruses and their temporal and spatial distribution in the cotton growing areas of Pakistan will be discussed.

GENETIC DIVERSITY OF RICE TUNGRO SPHERICAL VIRUS IN TUNGRO-ENDEMIC PROVINCES OF THE PHILIPPINES AND INDONESIA

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Coat protein regions 1 and 2 of rice tungro spherical virus (RTSV) were amplified from total RNA extracts of serologically indistinguishable field isolates from the Philippines and Indonesia and using reverse transcriptase polymerase chain reaction (RT-PCR). Digestion with Hind III and Bst YI enzymes differentiated the amplified DNA products into eight distinct coat protein genotypes. These were then used as indicators of virus diversity in the field and intra- and inter-site diversities were determined over three cropping seasons. In each of the sites surveyed, one or two main genotypes prevailed together with other related minor or mixed genotypes that did not replace the main genotype over time. In the Philippines, the cluster of genotypes among the sites was similar, however, in Indonesia, genotypes were clustered according to location. In North Cotabato, Bali and Subang, the genotype combination was stable over 2-3 seasons. Phylogenetic studies confirmed the classification of the different genotypes based on the RT-PCR and restriction analysis and showed that RTSV field populations could comprise isolates with a different evolutionary history at a single site.

MOLECULAR EPIDEMIOLOGY OF CUCUMBER MOSAIC VIRUS AND ITS SATELLITE RNA

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Cucumber mosaic virus (CMV) is endemic in horticultural crops all over Spain. To characterize the genetic structure of CMV populations, about 300 isolates, representing 17 outbreaks (i.e. sub-populations) in different crops, regions and years, were compared. Genetic analyses of CMV isolates were done by ribonuclease protection assay (RPA) of cRNA probes representing RNA 1, RNA 2 and both ORFs in RNA 3. All isolates belonged to three genetic types: sub-group II, and two types of sub-group I (IA and IB). Genetic types did not cluster according to geography, host plant species, or year of isolation. The genetic composition of the 17 sub-populations varied randomly. Thus, CMV in Spain shows a metapopulation structure with local extinction and random recolonization from local or distant virus reservoirs. The frequency of mixed infections, and of appearance of new genetic types by genetic exchange was also estimated.

About 30% of CMV isolates were supporting a satellite RNA (satRNA). The frequency of CMV + satRNA isolates differed for each subpopulation, being about 100% in Eastern Spain in 1990 and decreasing to 0% in distant regions and subsequent years. Molecular analyses of satRNA isolates show a single, undifferentiated population. Thus, CMV-satRNA population shows a different structure and dynamics as compared to CMV. This indicates that CMV-satRNA has spread epidemically on the extant virus populations from an original reservoir in eastern Spain.

BIODIVERSITY OF POPULATIONS OF CUCUMBER MOSAIC VIRUS IN ITALY BEFORE AND AFTER VIRUS OUTBREAKS IN 1988

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Cucumber mosaic virus (CMV) is the type species of the genus *Cucumovirus* in the family *Bromoviridae* and is recognised worldwide as a threatening pathogen of many commodity crops Strains of CMV can be divided into two sub-groups, I (or WT) and II (or S), on the basis of their sequence similarity and serological relationships.

Since 1988, the economic importance of CMV in the Mediterranean basin correlates with recurrent outbreaks in canning tomato crops In addition to the well known fern leaf /shoestring and tomato necrosis (synonym: tomato lethal necrosis) diseases, tomato fruit necrosis, a previously unrecorded disorder, was observed during CMV epidemics.

In the field, the symptoms consist of more or less extended internal necrosis of the fruits. Usually, the plants are vigorous and do not show symptoms on the foliage, except for occasional mild discolourations of the leaves. Sometimes, however, the plants show also the fern leaf/shoestring condition typically induced by ordinary CMV strains. Fruit tissues affected by necrosis are the mesocarp and particularly those surrounding the base of the pedicel. Externally, brown soft blotches correspond to altered tissues.

In Italy, the fruit necrosis disease was constantly associated with a sub-group I CMV strain (CMV-Tfn) which supports a 390-ribonucleotide satellite named Tfn-satRNA. This satellite RNA was not present in plants showing both fruit necrosis and fern leaf/shoestring. Its occurrence was apparently influential in determining the fruit necrosis condition, but prevented the appearance of phylimorphism on the leaves.

The analysis of the complete nucleotide sequence of CMV-Tfn and its comparison with the genome of other CMV strains places CMV-Tfn in the newly proposed subgroup IB strains of CMV originating from Asia and raises questions about its introduction to Italy.

A number of CMV isolates collected in Italy from different plant species before 1988 were analysed by RT-PCR with primers specific for CMV RNA 2 and RNA 3 followed by enzymatic digestion and results compared with those available for CMV isolates collected and characterized after the epiphytotics in 1988. Preliminary results indicate that relevant appearance of subgroup IB strains of CMV in cultivated crops in Italy was concomitant with epiphytotics in 1988.

VARIATION AMONG BOTH APHID CLONES AND VIRUS ISOLATES REGULATE POTATO LEAFROLL LUTEOVIRUS TRANSMISSION BY VECTORS OF THE *MYZUS PERSICAE* GROUP.

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Three-dimension epidemiological models rarely account for biological and genetic characteristics of their interacting components (*i.e*. virus, vectors, plants), nor do they account for the variation affecting them. Moreover, the taxonomic status of the vector *Myzus persicae*, is currently ill-defined (confusion between *M. persicae*, *Myzus antirrhinii* and *M. Nicotianae*). Transmission to *Physalis floridana* of a highly- (HAT) and a poorly- (PAT) aphid transmissible PLRV isolate (CU87 and 14.2, respectively) was compared under controlled conditions, using 15 yellowish-to-green clones initially known as *Myzus persicae* (Mp) and 2 of *Myzus nicotianae* (Mn), differing by their origins and sampling dates. All the clones transmitted the HAT isolate very efficiently (> 90%), yet unexpected broad variation in the PAT strain transmissibility was obtained. It was low for 15 clones (0% for 3 of them and from 2.5 to 26% for 12) and much higher for two others (Mp18=57% and MnLCSA=71%). Transmission rates were shown to be stable over time and did not depend on the origin of *P. floridana* source plants (cuttings or aphid-inoculated seedlings). The lowest % transmissions (< 10%) were mostly observed with the "oldest" clones (*i.e*. collected in the late 1960s or early 1970s) whereas the highest one was due to one of the two *M. nicotianae* clones.

Therefore, a genetic characterization of 27 clones (including the 17 above-mentioned) of the *M. persicae* group currently cultured was done. First, chromosomal races were identified with 2n= either 12, 12T (autosomal translocation A1,3), 13 or 14 (1 or 2 autosomal dissociations) chromosomes. The two latter karyotypes thus showed that *M. antirrhinii* (Ma) was present, comprising all the "oldest" clones. Secondly, the type (E4, FE4 or Susceptible) of the corresponding esterase resistance genes was examined by CAPS and their expression, as assessed from their methylation status, yielded two patterns corresponding to the two variants identified from allozyme analysis (EST, Phe-Leu, AAT systems). Moreover, the analysis of 2 allozyme and 9 microsatellite

loci discriminated 12 different genotypes and showed that *M. nicotianae* clones are very close to those of *M. persicae*, whereas *M. antirrhinii* ones are more distant, sharing few allozyme and microsatellite alleles.

As expected, variation in the transmissibility of isolate 14.2 could not be correlated to genetic diversity as revealed by neutral markers, even though all Ma clones were low transmitters which could show that overall they likely possess different genetic properties potentially involved in this poor vector capacity. It is concluded that since all the aphid clones of the *M. persicae* group can concurrently be both good vectors of some PLRV strains and poor vectors of another, low transmissibility cannot be attributed only to changes in viral particles alone or to the aphid clones on their own: the transmission process and its specificity must depend on interactions between both components.

BIOLOGICAL AND MOLECULAR CHARACTERISATION OF CLONES OF *MYZUS PERSICAE* AND *MYZUS ANTIRRHINII* WITH DIFFERENT EFFICIENCIES FOR POTATO LEAFROLL VIRUS TRANSMISSION

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Myzus persicae has long been recognised as the most efficient vector among the few aphid species reported to transmit potato leafroll virus (PLRV). Clones of *M. persicae* are known to vary in their transmission efficiency, but understanding the genetic basis of this variation has been limited by a lack of biochemical markers. Aphids resembling *M. persicae* were collected in 1994-1998 from potatoes and brassicas in Scotland, and clones derived from these aphids were examined using a novel rDNA fingerprinting technique that revealed distinctive patterns between clones. A large proportion of the clones appeared to be identical with a Scottish clone of *M. persicae* (Mp1S) that has been maintained for virus transmission studies at SCRI, Dundee since 1977. This clone fingerprint was not detected in samples of *M. persicae* from England, France or USA. In preliminary tests, some dark green clones transmitted PLRV more efficiently than did clone Mp1S. They were subsequently identified by karyotyping and morphology as *Myzus antirrhinii*, a closely related species not hitherto reported from field crops. Seven additional clones of *M. antirrhinii* from England and France were also found to be very efficient PLRV vectors. The rDNA fingerprints of all the *M. antirrhinii* clones were characterised by high molecular weight bands that were not present in *M. persicae*.

The transmission efficiencies of 12 clones of *Myzus* spp. were compared in tests using aquisition access periods of 24h or 48h on excised leaves of PLRV-infected *Physalis floridana* plants. Four clones of *M. persicae*, including Mp1S, transmitted PLRV to less than 30% of *Nicotiana clevelandii* test plants; the rest, including seven *M. antirrhinii* clones, transmitted PLRV to 68-91% of the test plants. Similar differences in transmission rates between efficient and inefficient clones were found when aphids were fed through membranes on purified PLRV. The epidemiological significance of variations in PLRV transmission efficiency, and biological factors, such as rates of alate production and obligate anholocycly, will be discussed in relation to variations in the annual incidence of PLRV in Scottish seed potato crops.

POSTER PRESENTATIONS

FIRST REPORT OF TOBACCO MILD GREEN MOSAIC TOBAMOVIRUS IN EGGPLANT AND PEPPER CROPS IN SPAIN

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Samples of greenhouse-grown egg-plants (*Solanum melongena* L.) with symptoms of stunting, foliar mosaic and fruit deformation were collected in Almería (Southeastern Spain) in the Spring of 1996. Negative staining of sap from these samples revealed tobamovirus particles. A field isolate from egg-plant (96/21) was purified after propagation in *Nicotiana clevelandii* plants. Inoculation of egg-plants with purified virions of isolate 96/21 reproduced the field symptoms. The host range of this tobamovirus (96/21) was studied and found to be similar to that of tobacco mild green mosaic virus (TMGMV). The relation between isolate 96/21 and TMGMV was confirmed by sequencing several cDNA clones obtained by random priming of viral RNA. The sequence homology between 96/21 and TMGMV was *c.* 98% in the clones analyzed. Serological characterization of isolate 96/21 was performed after obtaining a polyclonal antiserum to this isolate. ELISA tests showed that 96/21 was closely related to several TMGMV isolates included in the analysis. The U2 strain of TMGMV was the closest isolate to 96/21 and the U5 strain and other Spanish TMGMV isolates from *N. glauca* were less related to 96/21. The antiserum developed for 96/21 did not react strongly with other tobamoviruses such as U1-TMV or PMMoV. The result constitutes the first report of TMGMV naturally infecting egg-plant crops. In the winter of 1997 we also collected in the same area samples of greenhouse-grown pepper plants that were found to be infected with TMGMV and, in some cases, doubly-infected with TMGMV and pepper mild mottle tobamovirus. TMGMV had been reported previously in pepper crops in other areas, but this is the first record of this tobamovirus in pepper in Spain.

GENETIC DIVERSITY AND BYDV-TRANSMISSION EFFICIENCY OF *RHOPALOSIPHUM PADI* GENOTYPES

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Molecular biological methods, such as RFLP, PCR or SSR, are helpful and effective tools to differentiate aphid genotypes when used in combination with their morphological and biological characteristics. In RAPD-marker analyses individual aphids collected by suction trap and yellow pan trap in Aschersleben, Germany, exhibited a large genetic diversity within one local aphid population.

For further studies of the genetic variability of *Rhopalosiphum padi* six cultures from geographically different regions in Germany (D1 and D2 - Aschersleben; D3 - Rostock), the Czech

Republic (CZ1 - Prague), Bulgaria (BG1 - Sofia) and New Zealand (NZ1 - Christchurch) were investigated. They could be classified into three groups according to their fragment patterns. D1, NZ1, CZ1 and BG1 were quite similar, whereas D2 and D3 differed from each other and in comparison to the first group. These results show that genotypes from a geographically limited area (Germany) can exhibit greater differences than those from more distant locations (D1 and NZ1). To prove, if the genetic heterogeneity within one population is possibly as high as or higher than between them, more detailed marker experiments with a larger number of aphid individuals from the different origins should be made.

The serial transmission tests carried out under controlled conditions in climatic chambers (20 ^{o}C, 16 h photoperiod) demonstrated no differences for BYD-PAV. Infection rates were 100 %. Infected plants developed conspicuousg symptoms, especially dwarfing.

Significant differences between the aphid genotypes were observed for BYD-RPV. D3 and NZ1 were the most effective vectors with *c*. 90 % infection rate and the most conspicuous symptom expression followed by D1, BG1, D2 and CZ1 with only medium-strong symptoms. D3 and NZ1 were also the only genotypes which could transmit PAV (detected by DAS-ELISA) from a BYD-MAV/PAV-mixture.

Sitobion avenae and *Rhopalosiphum maidis* were also studied for their efficiency in transmitting different BYDVs. *S. avenae* gave the highest infection rate (50 %) for transmission of MAV (detected by DAS-ELISA) out of the BYD-MAV/PAV-mixture. BYD-PAV and BYD-RPV were transmitted at infection rates of 25 % and 5 %, respectively. *R. maidis* did not transmit any of the viruses tested.

Up to now no molecular marker could be found correlating with the differences detected in BYDV-transmission.

SEROLOGICAL, MOLECULAR AND BIOLOGICAL VARIABIILITIES OF RICE YELLOW MOTTLE VIRUS (RYMV) ISOLATES FROM IVORY COAST

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Rice yellow mottle virus (RYMV) causes the most important disease of rice so far reported from Africa. First described in 1966 in East Africa, RYMV causes a major problem of rice cultivation especially in lowland area. The importance of the disease appears to be increasing over the years in West Africa. Infection has been reported in several countries. Under natural conditions, RYMV is transmitted by chrysomelid beetles, but it is also mechanically transmissible. Severe yield losses ranging from 20 to 100% caused by RYMV have been recorded in several rice cultivars. Despite its devastating nature, little is known on the epidemiology of the disease. There is little information on the variability of RYMV isolates though this can play a key role in developing control

measures and resistant varieties. Differences in pathogenicity of RYMV isolates originated from closely related agroecological zones were reported from Burkina Faso. Serological differences between one isolate from Ivory Coast (West Africa) and one from Kenya (East Africa) were first reported. Latter, serological diversity of a collection of five isolates, each one from different African county, was studied Three serogroups were defined but there was no geographical basis to their distribution. By contrast, in Burkina Faso and in Mali (West-Africa), the three serogroups found were tentatively linked to their ecological origin and pathogenicity. In this study, we assessed the serological, molecular and biological variabilities of RYMV isolates from all main rice-growing regions of Ivory Coast.

Serological Variability: Serological variability of isolates of RYMV collected in Ivory Coast was assessed by immunological tests with polyclonal and monoclonal antibodies. Serological variability among isolates was apparent and the presence of two distinct serotypes (named S1 or S2) was established. The Sl isolates had common epitopes that were absent in S2 isolates, and inversely they lacked epitopes shared by S2 isolates. There was no evidence of S1 and S2 in mixtures, although both Sl and S2 isolates were found in all rice-growing regions of Ivory Coast, sometimes in fields only a few kilometres apart. Geographical distribution of the two serotypes was different. Overall, Serotype S2 was more prevalent than S1 in Ivory Coast, and was in majority in the centre and the south of the country. By contrast, S1 was more widespread in the north of Ivory Coast. In neighbouring countries, isolates of the S1 serotype were found, but none of the S2 serotype. In other African countries, in East-Africa especially, three additional serological patterns not found in Ivory Coast were detected.

Molecular Variability: RT-PCR amplification of genome regions with the CP gene of S1 and S2 isolates was contrasted. The S2 isolates were successfully transcribed and amplified by RT-PCR using the primers developed against an S2 isolate from Ivory Coast. Under these conditions, S1 isolates were not amplified. Another set of primers was developed which allowed amplification of a genome region with the CP gene of both Sl and S2 isolates, and also of isolates of the serotypes found outside Ivory Coast. This separation of the RYMV isolates based on their reaction to the two sets of primes was confirmed by nucleotide sequence analysis of the coat protein (CP) gene. Difference between S1 and S2 isolates is based on 162 nucleotides of the 720 analysed.

Biological Variability: Based on the response of rice varieties and landrace cultivars inoculated with RYMV isolates SI and S2 from Ivory Coast, different pathogroups were apparent. Both S1 and S2 isolates have the same virulence. The separation of the isolates according to their pathogenicity is not related to their serological properties. Biological competition between SI and S2 isolates was apparent, as S2 isolate was predominant after simultaneous co-inoculation of rice plants.

EPIDEMIOLOGICAL FACTORS THAT DETERMINE THE EVOLUTION OF THE SATELLITE RNA OF CUCUMBER MOSAIC VIRUS.

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Isolates of cucumber mosaic virus (CMV) infecting tomato crops in E. Spain were found to frequently support satellite RNAs (satRNAs). The appearance of CMV-satRNA in the field in the late 1980s was associated with a new syndrome of systemic necrosis. The frequency of CMV-infected necrotic plants decreased from 1989 on, until its disappearance in 1992, but satRNAs were still associated with CMV until 1994. We have shown that the disappearance of tomato necrosis was due to the substitution of necrogenic types of CMV-satRNA by non-necrogenic ones. We have analyzed different epidemiological parameters that could explain the observed evolution of virulence in CMV-satRNA populations. Infectivity assays showed that necrogenic and non-necrogenic satRNAs were equally infectious to tomato plants. Necrogenic satRNAs accumulated to higher levels and were encapsidated more efficiently than non-necrogenic ones. Furthermore, competition experiments showed an advantage for necrogenic satRNAs. In contrast, necrogenic satRNAs decreased the accumulation of CMV more than non-necrogenic ones. Quantitative estimates of these various factors show that the evolution of virulence in CMV-satRNA populations can be explained by trade offs among those that favor necrogenic types (accumulation in tomato leaves, efficiency of encapsidation), and those that favor non-necrogenic ones (higher levels of helper virus).

NUCLEOTIDE SEQUENCE OF THE 3'-TERMINAL REGION OF AN ISOLATE OF POTATO VIRUS Y INDUCING VEINAL NECROSIS IN PEPPER (PVYnnp)

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The nucleotide sequence of the 3' terminal region (7240 nucleotides) of an isolate of potato virus Y (PVY) RNA, recovered from pepper (*Capsicum annuum*), showing veinal necrosis and named PVY-nnp, has been determined in southern Italy. Previously biological data showed that PVY-nnp differs from all the other PVY strains including isolates normally infecting pepper, therefore it does not belong to any of the PVY^O or PVY^N groups.

The comparison of the nucleotide and amino -acid sequences of the PVYnnp analysed genes showed that the PYVnnp is closer to PVY^O than PVY^N isolates.

MYZUS CERASI VECTOR OF THE SWEET CHERRY STRAIN OF PLUM POX POTYVIRUS

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Plum pox potyvirus (PPV) is the causal agent of sharka disease in stone fruits (plum, peach, apricot and, as found recently, sour and sweet cherry). On the basis of biological, serological and molecular characteristics PPV isolates have been grouped in three different sub-groups: M, D and C. M group isolates seem to spread more easily than those of D and to cause more severe infections in peach orchards. C group isolates have been recovered only from sour and sweet cherry and seem to be confined to these species. PPV is spread naturally by aphid vectors in a non-persistent way. The sweet-cherry strain of PPV (PPV-SwC) has been transmitted by *Aphis fabae* and *Myzus persicae*. In this work we demostrate that *M. cerasi* is another vector of PPV-SwC.

M. cerasi aphids were reared on healthy sweet cherry plants, starved for 3 to 5 hours, allowed to feed for 1 to 3 minutes on infected sweet cherry plants and then used for IC-RT-PCR or transferred to healthy sweet cherry plants. The insects were killed one hour later with an insecticide. Four months after inoculation the plants were checked by RT-PCR using samples from new leaves.

The amplification products obtained by IC-RT-PCR of homogenized aphids were of the expected size. The same result was obtained by RT-PCR of TNA extracts from the tested plants. Non-specific amplified products were obtained when less than five aphids were used as samples, with samples derived from non-viruliferous aphids or with TNA obtained from healthy plants. These results were confirmed by Southern blot hybridization analysis in which the amplified products were detected by specific ribo-probes.

This investigation demonstrates that PPV-SwC is transmitted by *M. cerasi* from cherry to cherry plants and also the successful use of PCR technology to detect PPV directly in aphids. It is concluded that *M. cerasi*, which according to some authors is not a vector of M and D isolates, could have had a role in selecting and spreading PPV-SwC in nature.

GENETIC CHARACTERIZATION OF TOBACCO POTATO VIRUS Y STRAINS IN SPAIN

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Potato virus Y (PVY) is an important pathogen, mainly of cultivated solanaceous vegetables, such as potato, pepper, tomato, tobacco, and others. It is naturally transmitted by several aphid species in a non-persistent manner. PVY isolates have been differentially classified depending on their original host. The isolates that have received more attention are those derived from potato and pepper. Potato isolates have been classified into Y^O, Y^N and Y^C strains, using a combination of criteria including symptomology, serology and host-plant resistance responses. For pepper-isolates, a

pathotypic classification has been proposed, based on their ability to overcome several resistance sources. Tomato PVY-isolates have been characterized only superficially. We have recently classified a collection of tomato-PVY isolates using genetic criteria [Blanco-Urgoiti *et al*., in preparation]. Tobacco isolates have been classified according to the symptoms induced in flue-cured tobacco cultivars resistant or susceptible to the root-knot nematode, but a good strain classification is lacking for them.

The use of different criteria has hampered a clear classification of PVY isolates into strains. In our laboratory, a host-independent classification based on RFLP patterns of the coat protein gene, after immunocapture-RT-PCR, has been developed. This approach allows to determine genetic strains of PVY. We have proposed the term "restrictotype" to define each isolate taxonomically. This rapid molecular typing approach grouped PVY isolates in three main clusters: potato PVY^N, potato PVY^O and non-potato isolates, including two pepper, 4 tobacco and one *Datura* spp. isolates.

Due to the lack of a genetic classification of tobacco-isolates, we have undertaken the genetic characterization of PVY isolates collected from tobacco-fields of different locations in C·ceres, Spain, during the summer of 1998. We collected plants with symptoms of PVY, that were confirmed by ELISA. Extracts of PVY-infected tobacco plants were subjected to specific immunocapture-RT-PCR. The results obtained for this first year of the analysis will be presented.

TOWARDS AN ELUCIDATION OF THE GENETIC STRAINS OF TURNIP MOSAIC VIRUS

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Turnip mosaic virus (TuMV) is a potyvirus. It is a very widespread pathogen, mainly of cultivated crucifer crops. It has been considered within the five most damaging viruses of horticultural crops, where it is often found in association with other viruses including cauliflower mosaic viruss. It is also able to infect systemically the widely used "model" species *Arabidopsis thaliana*. The virus shows a high level of variability. For example, up to twelve different pathotypes have been described, using a combination of two resistance sources. From a serological standpoint, several monoclonal antibodies have been produced that are also able to differentiate TuMV isolates. Finally, using a set of differential hosts, several virus biotypes have been proposed.

Several TuMV full-length sequences have been published, and a larger number of coat protein gene sequences are available in databases. However, a genetic-based classification of TuMV isolates into strains has not been proposed. We are analyzing a large collection of TuMV isolates maintained at HRI, using a restrictotype approach. The results obtained so far, indicate strongly that true genetic strains can be proposed, and that the host plays an important role in their establishment. A genetic classification of TuMV isolates into strains should be a valuable tool for future epidemiological studies.

NATURAL OCCURRENCE OF CUCUMBER MOSAIC CUCUMOVIRUS

AND ITS SATELLITE RNA ON PEPPER CROPS IN ARGENTINA

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The natural occurrence and distribution of cucumber mosaic virus (CMV sub-groups and associated satellite RNAs in the main pepper-growing regions of Argentina, both in open field and protected crops, has been determined for the first time. Both viral sub-groups were found in a clear-cut distribution pattern depending on the different geographical origins, but sub-group I appeared largely as the predominant population encountered and no sub-group mixed infections were detected. Satellite RNAs were found associated with most isolates irrespective of their origin or helper sub-group and they all showed a close relation among each other and close relation to CMV-Y satellite RNA, suggesting similar evolutionary histories. A new method has been used for CMV sub-group differentiation that is based on specific restriction endonuclease digestion of PCR-amplified viral capsid protein gene cDNAs and relies on the presence/absence of restriction enzyme recognition sites conserved among published isolates, which lets each enzyme be considered separately for sub-grouping and each independent restriction event corroborates the other enzyme result. This fact makes it more reliable than previous similar methods. The result of the CMV survey has shown that the virus has become prevalent and causes a major problem of pepper, having a severe and increasing impact in all pepper cropping regions and progressively displacing pepper severe mosaic virus (PeSMV) and potato virus Y (PVY). The level of damage caused by CMV in the different growing regions emphasises the importance of factors such as temperature, plant genetic variability and the effect of cultural practices.

IMPROVEMENT OF DIAGNOSTIC METHODS FOR THE DETECTION OF VIRUSES AND VIROIDS OF PHYTOSANITARY IMPORTANCE

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In the last years, new regulations, either at international or national level, have been set up with the aim of regulating the commercialisation of vegetative propagative material and indicating the sanitary and qualitative requirements for the safe movement of germplasm around the world. Of course the most dangerous pathogens, essentially the systemic ones (viruses, phytoplasmas and viroids) must be excluded from material being transported, so that different studies are aimed at improving and optimising methods able to detect them. Here, we report our experience in establishing the most reliable and sensitive protocols to exclude the presence of three pathogens of quarantine importance that are causing severe problems in the cultivation of fruit trees and potato in Italy.

Plum pox potyvirus. The diagnosis of PPV is still considered one of the most important aspects of the ìSharka" problem. In fact, different studies demonstrated an uneven distribution of the

virus in infected trees, complicating PPV detection. Biological, serological and molecular assays were developed successively in order to obtain sensitive and efficacious PPV detection techniques. The polymerase chain reaction (PCR) technology seems to be particularly promising and can be considered the most sensitive and reliable laboratory assay. Sample preparation for the extraction of the viral RNA is still a fundamental step in PCR. In order to find a less time-consuming and efficient method, we compared different protocols to extract viral RNA to process in RT-PCR. The evaluated procedures have, as a common characteristic, the possibility to extract the RNA from a small amount of plant tissue avoiding the use of organic solvents in the extraction buffer. Three viral RNA preparation methods were applied: immuno-capture (IC), using a specific antiserum; silica-capture (SC), using a non-specific matrix, and a simple and rapid extraction method (REM). Each procedures was followed by one-step RT-PCR. From the results obtained we routinely perform PPV detection as follows: REM-PCR in spring and summer time no matter the host or the matrix; IC-PCR in the other periods using leaves and bark from trees to be tested.

Peach latent mosaic viroid. PLMVd is widespread in Italy, either in symptomatic or in symptomless plants. Some isolates induce mosaic symptoms on leaves, delay foliation, flowering and maturity, calico and malformation of fruits with cracked sutures and enlarged pits, bud necrosis and, in the more severe cases, early decline of the trees. Until a few years ago detection of PhMVd was based mainly on cross-protection tests using GF305 peach seedlings. We compared the results obtained in the diagnosis of PLMVd performed with biological indicators and molecular techniques ("spot" and "tissue blot" hybridization using non-radioactive probe and RT-PCR) starting from different matrices (leaves, buds and bark) collected from infected and healthy peach plants. On the basis of the results obtained, tissue blot hybridization technique has been chosen for routine and large scale diagnosis In fact, with this technique, it is possible to detect the viroid during all the vegetative stages (including winter) utilising different parts of the plant (including bark and buds).

Potato virus Y-NTN. In 1997, potato tuber necrotic ringspot disease, caused by a variant of PVY^{N}, appeared in Italy causing severe damages in ware potato. This variant produces symptoms on the tubers as sunken and necrotic rings and areas that are particularly evident after storage. Moreover, PVY^{NTN} causes degradation of tuber starch into simple carbohydrates that are undesirable for processing potato into chips. The identification of PVY^{NTN} is not possible serologically; in fact, no amino-acid sequence differences are present in the coat protein of this isolate compared with the other members of PVY^{N} strain group. By sequencing the genome of a PVY^{NTN} isolate, specific primers are available to be applied in polymerase chain reaction for diagnosis. To investigate the quality of seed potato and the incidence of the PVY^{NTN} in ware potato in Italy, RT-PCR with total RNA extraction, IC-RT-PCR and one step IC-RT-PCR were performed in our laboratory. In our experience, RNA extraction is required when dormant tubers have to be tested. IC-RT-PCR is reliable and sensitive for sprout and leaf testing. For massive and routinely PVY^{NTN} indexing one-step IC-PC-RT is very useful, rapid and reliable.

In conclusion, in our experience, molecular approaches are very helpful in improving the detection of dangerous pathogens and should be adopted more frequently for mass scale diagnosis to guarantee the health status of propagation material.

ANALYSIS OF THE INCIDENCE OF MAIZE-INFECTING POTYVIRUSES IN MIDDLE GERMANY

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In Middle Germany maize is found to be infected by sugarcane mosaic potyvirus (SCMV) as well as maize dwarf mosaic potyvirus (MDMV). However, SCMV was dominant with a percentage of > 99 % in the majority of the last years. In contrast, the MDMV is the most prevalent virus in southern European countries.

From an analysis of sequence data obtained for the coat protein gene of four German isolates and a comparison with corresponding data for strains of SCMV concluded that the German isolates represent a separate 'cluster' within the SCMV. The German isolates investigated showed a high degree of sequence homology among each other. To a great extent they correspond also with regard to the size of the coat protein as well as to serological and biological features. In the N-terminus of the coat protein German isolates revealed a *Pst* I restriction site which was absent in all other strains of SCMV yet sequenced. Using specific primers and a subsequent digestion with *Pst* I the differentiation of an independent strain "SCMV-GER" was possible.

Comprehensive investigations concerning the ecology resulted in the clarification of epidemiological infection cycles. According to our findings several winter annual species of the genus *Bromus* as well as the perennial *Roegneria canina* (L.) (Nevski) can serve as virus source for the infection of maize crops. In this connection the detected seed transmission seems to be unimportant.

Regarding the natural transmission of both SCMV and MDMV by vectors *Rhopalosiphum maidis* (Fitch) proved to be the most effective vector, followed by *R. padi* (L.). Up to now *R. maidis* has never been found in Middle German maize crops. Therefore, *R. padi* is probably the vector of greater importance. *Metopolophium dirhodum* (Walk.) compensates for a low effectiveness by its high abundance.

Since 1990 in maize crops the occurrence of potyviruses has been decreasing continuously. Weather conditions and general changes in the cultivation of maize are the reasons for this development. Nowadays in almost every case maize is cultivated as a main crop and sown early. The resistance level has also been improved.

DEVELOPMENT OF AN ISOTHERMAL AMPLIFICATION METHOD TO DISTINGUISH CUCUMBER MOSAIC VIRUS ISOLATES

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An isothermal amplification method for nucleic acids, NASBA, was developed to detect cucumber mosaic virus (CMV), a cosmopolitan virus. CMV has a tripartite RNA genome, and the numerous reported isolates have been grouped in two sub-groups (named I and II) according to distinct biological, serological and nucleotide sequence characteristics. To amplify viral RNA, a set of four different primers was selected in conserved areas of the 3a open reading frame sequence, using an alignment of 23 CMV RNA 3 sequences. The best primer combination was used for the remaining experiments. The NASBA reaction products were visualized after electrophoresis by ethidium bromide. The specificity of the assay was tested by Northern blot analysis with two CMV sub-group-specific biotin-labelled oligonucleotide probes and the Amersham's ECL chemiluminescent system. To test the sensitivity of the assay, *in vitro* RNA was prepared by transcription of PCR products obtained by using a sense primer with a 5'-T7 promoter sequence. When *in vitro* RNA obtained from two isolates of sub-group I and II, respectively, were assayed, 10^3 copies of RNA could be detected easily in each case, indicating the high sensitivity of the method. To test the feasibility of the assay with leaf samples, the method was coupled to immunocapture in tubes of CMV virions present in leaf samples followed by NASBA and hybridization with a specific probe. Thus, it was possible to detect all samples assayed and to discriminate between sub-groups I and II.

EPIDEMIOLOGY AND GENETIC DIVERSITY OF BEAN POD MOTTLE COMOVIRUS

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Bean pod mottle virus (BPMV) is an economically important pathogen of soybeans in several of the southern, midwestern and eastern states in the USA. Presently, soybean cultivars with resistance to BPMV are not commercially available. Therefore, an understanding of BPMV epidemiology is critical to disease management. The primary sources of BPMV inoculum in soybean fields early in the season have not previously been critically examined. In this study, the roles of overwintering bean leaf beetles and seeds from infected plants in the epidemiology of BPMV were evaluated. Although BPMV antigen was readily detected in the regurgitants from naturally overwintered beetles, none of them transmitted the virus to healthy soybean seedlings. Similar results were obtained with virus-carrying beetles that were exposed to artificial overwintering conditions. The overwintered beetles, however, regained their ability to transmit BPMV when allowed to acquire BPMV from infected plants. Regurgitants from overwintered beetles were infectious by mechanical inoculation and viral RNAs extracted from such regurgitants showed no apparent changes in their integrity. Both large and small capsid proteins (CPs), however, exhibited limited proteolysis. Of particular interest was the cleavage of a 10 kDa peptide that, based on size

and partial sequence, is predicted to be carboxy coterminal with the large CP. Coupling the sequencing data of the N-terminus of the 10 kDa peptide with the known 3-dimensional structure of BPMV, it was possible to localize the cleavage site of the 10 kDa peptide at the surface of the capsid. We are currently investigating whether cleavage events are related to the loss of beetle transmissibility. Using seedling grow-out tests and ELISA, we were not able to demonstrate seed transmission of BPMV in seed lots from plants in at least two fields with near 100% BPMV incidence. Future plans involve investigating the role of the perennial weeds *Desmodium* sp. as sources of BPMV. Beetles have been shown to transmit BPMV to soybean plants following acquisition from BPMV-infected *Desmodium* plants. In a related study, Northern hybridization analyses using cloned cDNA probes to BPMV genomic RNAs 1 and 2 indicated the occurrence of at least two distinct sub-groups of BPMV strains.

THE USE OF POLYCLONAL AND MONOCLONAL ANTIBODIES DIRECTED AGAINST POTYVIRUS Y AND INSITU DETECTION OF THIS VIRUS ON TOBACCO LEAF EPIDERMIS

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Potato virus Y is the type member of potyvirus group. It infects solanaceous causing devastating damages on culture. Potyvirus genomic positif strand RNA encodes a polyprotein of about 350 kDa that is autocatalytically cleaved generating the different PVY proteins.

A panel of monoclonal antibodies (mAbs), directed against partially purified nuclear inclusion fraction (NI), were prepared. Three mAbs from this panel were characterized. They were directed against nuclear inclusion a (NIa), cytoplasmic inclusion (Cl) and capsid (CP) proteins.

These three mAbs, as well as the anti-NI polyclonal serum, were used to develop a rapid and easy method for *insitu* immunodetection of the virus Y proteins on infected leaf epidermis. The virus detection was performed using immunofluorescence and immunocytochemical techniques.

The results confirm the specific localization of cytoplasmic inclusion and capsid proteins in the cytoplasm. However, the nuclear inclusion NIa was detected both in the nucleus and in the cytoplasm.

SESSION 3

WHITEFLY-ASSOCIATED PROBLEMS OF VEGETABLE CROPS

ORAL PRESENTATIONS

OCCURRENCE OF CUCURBIT YELLOW STUNTING DISORDER VIRUS (CYSDV) AND BEET PSEUDO-YELLOWS VIRUS IN CUCURBIT CROPS IN SPAIN AND TRANSMISSION OF CYSDV BY TWO BIOTYPES OF *BEMISIA TABACI*

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The relative occurrence in Spain of two whitefly-transmitted closteroviruses causing similar yellowing diseases in melon and cucumber greenhouse crops was studied. Based on a RT-PCR assay, a 1994-1997 survey of Spanish greenhouses showed that the recently described *Bemisia tabaci*-transmitted cucurbit yellow stunting disorder virus (CYSDV) has displaced the *Trialeurodes vaporariorum*-transmitted beet pseudo-yellows virus (BPYV), a virus that was present in the area since the late 1970s. The CYSDV transmission rates by each of the two biotypes of *B. tabaci* present in Spain were compared. The results showed that the ubiquitous "B" biotype and the resident "Q" biotype (found in Spain and Portugal) transmitred CYSDV with similar efficiency. Melon germplasm containing a gene that confers resistance to CYSDV has been described in another laboratory. We are currently collaborating in determining the mode of action of the gene. Finally, experiments are in progress to monitor the colonization of host plants by CYSDV using RT-PCR detection and to compare the colonization pattern with the appearance of symptoms. Results will be useful to optimize sampling methods that will facilitate collection tissues with high virus titre in the field surveys.

WHITEFLY-TRANSMITTED VIRUSES INFECTING SWEETPOTATO IN THE UNITED STATES

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Although, sweetpotato (*Ipomoea batatas*) has been widely recognized to be infected with viruses, only one well characterized virus, sweetpotato feathery mottle virus (SPFMV), has been reported to occur widely in sweetpotato growing regions of the United States. Recently, a closterovirus, sweetpotato chlorotic stunt (SPCSV) and a geminivirus, sweetpotato leaf curl (SPLCV) have been reported in the United States. In 1994, sweetpotato samples were collected from various growers and experimental plots in Louisiana. Two virus isolates were obtained after whitefly

transmission experiments. The viruses were identified as SPCSV and SPLCV. Identification of these two viruses was conducted by PCR, molecular hybridization, electron microscopy, host reaction, and whitefly transmission studies. Due to the variety of *Ipomoea* species that occur naturally in Louisiana, host range studies with these two viruses were conducted by graft-inoculations. Field-collected *Ipomoea* species were tested for the presence of these two viruses as well as for SPFMV. Only one specie, *I. lacunosa*, was found naturally infected with SPLCV. In 1998, two whitefly species: *Bemisia tabaci* biotype B (sweetpotato whitefly) and *Trialeurodes abutilonea* (banded winged whitefly) were observed in low populations in sweetpotato fields in Louisiana. Transmission experiments were conducted using colonies of these two whitefly species. In general, single whitefly transmission experiments with SPCSV resulted in 10% transmission with *B. tabaci* and 5% with *T. abutilonea*. SPLCV was transmitted by *B. tabaci* at the rate of 3%. Transmission rates increased with and increase in the number of whiteflies used.

EXPANSION OF TOMATO-INFECTING CRINIVIRUSES INTO NEW AREAS

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Greenhouse culture of vegetables and ornamentals have increased the geographical range where members of the new whitefly-transmitted *Crinivirus* Genus are found. *Bemisia* whiteflies are normally restricted to the tropical and subtropical climates, however greenhouse culture has allowed *Bemisia* and *Trialeurodes* spp. whiteflies to survive where they would not normally occur. At least three distinct whitefly-transmitted bipartite criniviruses, tomato infectious chlorosis virus (TICV), tomato chlorosis virus (ToCV), and an unnamed crinivirus infecting tomato have been found, both in field and greenhouse-grown tomatoes. These viruses have wide host ranges and include ornamentals, weeds and agronomic crops, including sugar beet and potato. TICV has been found in California, North Carolina, Italy, and Taiwan, and ToCV in Florida, Louisiana, Colorado, and Taiwan, naturally infecting tomatoes, zinnia, petunia, and ranunculus. Although TICV is only transmitted by the greenhouse whitefly, (*Trialeurodes vaporariorum*), ToCV is transmitted by four whitefly vectors, including *T. vaporariorum, Bemisia tabaci* A and B biotypes, and the banded wing whitefly (*T. abutilone*). Both TICV and ToCV are considered to be semi-persistent in their vectors. TICV persists in the whitefly for four days, whereas ToCV persists one day in the vector. The third whitefly-transmitted crinivirus infecting tomato also is transmitted by the four vectors, and has been found in the Canary Islands. This virus is bipartite, and does not react with nucleic acid probes to either TICV or ToCV.

It is suspected that these viruses will be found in mixed infections with each other and with geminiviruses as well, as observed previously. Movement of viruses and viruliferous whiteflies in breeding material as well as international trade and increased greenhouse vegetable culture contribute to expansion of the natural range of these viruses.

RELATIVE IMPORTANCE OF TOMATO YELLOW LEAF CURL VIRUS-Is AND -Sr SPECIES IN INFECTIONS OF TOMATO IN SPAIN

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Tomato yellow leaf curl virus (TYLCV) was first reported in Spain in 1992 as the causal agent of tomato yellow leaf curl (TYLC) epidemics in tomato (*Lycopersicon esculentum* Mill.) and it is widespread in south and southeastern regions. Isolates of the TYLCV-Sr species were associated with TYLC symptoms. Recently, isolates of the TYLCV-Is species were also found to be involved in TYLC epidemics and were associated with the more severe symptoms caused in tomato. The presence and relative importance of both TYLCV species in successive epidemics in tomato has been studied in south and southeastern Spain. Disease progress curves were obtained for 1996, 1997 and 1998 epidemics in open field tomato crops from Málaga (southern Spain) and for 1997 in protected tomato crops in Almería (southeastern Spain) based on random samplings made weekly in three commercial fields per year. In the same years, tomato surveys were also made in the main tomato-growing regions of south and southeastern Spain. Digoxigenin-labeled DNA probes that specifically recognise TYLCV-Sr or -Is were prepared and samples were analysed by hybridisation of prints of leaf petiole cross sections on nylon membranes. Results will be presented that show clearly a rapid increase of the relative importance of TYLCV-Is in TYLC epidemics in Spain. Two aspects that could be involved in the differential spread of both viruses in tomato crops have been studied: first, the differential ability of local biotypes of *Bemisia tabaci* Gen. (biotypes B and Q) to transmit TYLCV-Sr and -Is isolates from Spain; second, the occurrence and relative importance of TYLCV-Sr and -Is in alternative hosts. Transmission experiments were done from tomato to tomato using two adult females per test plant, with acquisition and inoculation access periods each of 24 h. Experiments were performed in a growth chamber at 25 ºC day, 20ºC night and 16 h photoperiod. Results showed differences in transmission: both TYLCV-Sr and -Is are efficiently transmitted by biotype Q, similarly to transmission of TYLCV-Is by biotype B; whereas TYLC-Sr was transmitted at a significantly lower efficiency by biotype B. In addition, TYLCV-Is was found to be more prevalent in alternative hosts than TYLCV-Sr. The latter should be stressed especially for common bean (*Phaseolus vulgaris* L.), a species that is frequently used in crop rotations with tomato, in which high incidences of TYLCV-Is infections have been reported recently. The implication of these results in the epidemiology of TYLCV-Sr and -Is will be discussed.

MANAGEMENT OF GEMINIVIRUS EPIDEMICS OF FIELD-GROWN TOMATO IN FLORIDA AND THE DOMINICAN REPUBLIC

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Although known from Florida more than 100 years ago, *Bemisia tabaci* was not reported to infest tomato or many other vegetable crops such as cucurbits prior to 1986. The situation changed with the appearance, first on poinsettia, of the silverleaf whitefly, a new biotype later described as the species *Bemisia argentifolii*. Named for a squash disorder induced by nymphal feeding, high populations of silverleaf whitefly were soon seen on vegetables. In tomato, impact from feeding and sooty mould was quickly surpassed (in 1988) by appearance of a new disorder, irregular ripening, in

turn surpassed in impact a year later by a new geminivirus, tomato mottle (ToMoV). Losses and control costs during the 1990-1991 season were estimated at 141 million $US. A similar series of events occurred in the Dominican Republic except that indigenous geminivirus was supplanted by tomato yellow leafcurl virus in 1992-93, devastating the process tomato industry. A key component of management in both locations was the voluntary (Florida) or mandatory (DR) imposition of a crop-free period in summer to break the cycle of both virus and vector. In Florida, it was demonstrated that tomato was practically the only source of virus inoculum and that whitefly did not survive well on native weeds, due largely to natural biological control. However, early "plow-down" and prohibition of all vegetable production during summer was successful in the Dominican Republic, in spite of the presence of alternative weed hosts of TYLCV. This success demonstrated the predominant role of tomato as a source of TYLCV and of vegetables in general as whitefly sources. Widespread use of the insecticide imidacloprid in Florida greatly reduced whitefly populations to the extent that ToMoV has largely disappeared, although TYLCV is now posing a new threat. Imidacloprid was used much less in the lower input Dominican process tomato industry which relied more on protecting transplant production, planting schedules and host plant resistance. These experiences demonstrate the importance of a crop-free period for successful management of the whitefly/geminivirus complex.

THE EPIDEMIOLOGY AND MANAGEMENT OF TOMATO LEAF CURL VIRUS AND *BEMISIA TABACI* IN SOUTHERN INDIA

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Tomato leaf curl virus (ToLCV), transmitted by the whitefly *Bemisia tabaci*, is considered by tomato farmers in southern India to be their most important crop pathogen. ToLCV epidemics occur annually in the "summer months" (February-May) in this region and when infection occurs at an early stage of crop development, the disease can result in total yield loss.

ToLCV was detected in field-collected *B. tabaci* using a triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) as well as in weed species commonly found in Karnataka State. ToLCV from *c*. 61% of infected plants was transmitted successfully to tomato by *B. tabaci*. Weed species that are hosts of both the virus and the vector had averages of between 1.5-10.0 *B. tabaci* nymphs per plant, whereas the tomato plants had only 0.3 nymphs per plant. Additional data collected in the screen house and in the field showed that tomato is not a preferred host of south Indian *B. tabaci*, and that most of the ToLCV infection in the field arises from the movement of viruliferous *B. tabaci* adults into the crop. When tomato was grown for the first time in an entirely new area, ToLCV incidence reached *c*. 83% only 90 days after planting, demonstrating the importance of alternative host-plant species in this pathosystem.

The potential of several management technologies such as ToLCV-resistant tomato genotypes, nylon nets and mycopesticides were assessed in screening and field trials and a management approach based on an improved understanding of the *B. tabaci*/ToLCV/tomato system in southern India will be presented.

TOMATO BREEDING LINES DERIVED FROM *LYCOPERSICON HIRSUTUM* THAT ARE IMMUNE AND TOLERANT TO TOMATO YELLOW LEAF CURL VIRUS (TYLCV)

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Two TYLCV-resistant plants from accessions LA 1777 and LA 386 of the wild tomato species *Lycopersicon hirsutum* have been crossed. The resulting resistant F1 plants were crossed with the domesticated tomato *L. esculentum* and a series of selfings was performed. At each generation, individuals were selected for immunity (no symptoms and undetectable viral DNA) and tolerance (no symptoms but with detectable viral DNA), following controlled massive and repeated inoculations with viruliferous whiteflies. A stable BC1F4 line (designated 902) which does not segregate for immunity was obtained . This line does not support virus accumulation even after extensive whitefly-mediated inoculation of young seedlings. Whiteflies colonized the virus-immune plants but virus was undetectable by Southern blot and by PCR. Another stable BC1F4 line (dedesignated 908) was tolerant to the virus. Both lines have good horticultural characteristics and bear red fruits each weighing 80-120 g. Both lines do not need protection with nets and/or insecticides. In addition to immunity, line 902 showed extreme resistance to high temperatures, yielding many fruits when other cultivars were fruitless.

Analysis of segregation of susceptibility, tolerance and resistance during the BC1F1 to BC1F4 crosses indicated that tolerance is controlled by a dominant major gene and immunity by 2 to 3 additive recessive genes. Grafting onto infected susceptible *L. esculentum* indicated that TYLCV can infect and spread into the immune tissues: virus was present but the plant remained symptomless. Therefore we assume that immunity is the result of a block of virus replication and/or spread somewhere between inoculation during access feeding and transport to the phloem.

THE EFFECT OF TOMATO YELLOW LEAF CURL VIRUS ON NEW BREEDING LINES WITH HIGH LEVELS OF RESISTANCE TO THE VIRUS

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Tomato yellow leaf curl virus (TYLCV) is one of the most devastating viruses of tomatoes. The virus is a monopartite geminivirus, transmitted by whiteflies. The best way to reduce TYLCV spread is by breeding for resistance. Here we report new tomato breeding lines, which exhibit a very high level of resistance to the virus. To test the resistance level of these breeding lines, a field test was carried out. The new breeding lines TY172 and TY197 were compared to commercial TYLCV-resistant cultivars. Following inoculation the plants were transplanted in the field. Non-inoculated plants of the same cultivar or line served as controls. The inoculated plants were compared to the control, non-inoculated plants, in terms of total yield and fruit weight and number. Disease symptom development and virus accumulation in the inoculated plants were also monitored. There were substantial differences in the level of tolerance exhibited by the various cultivars and breeding lines, and TY172 and TY197 expressed the highest level of tolerance.

To further study the resistance displayed by TY172, infected susceptible scions were grafted onto healthy TY172 stocks. The grafted plants were tested for over three months, and during this time period the grafted TY172 plants did not develop disease symptoms, nor did they accumulate high levels of virus. In contrast, when infected susceptible scions were grafted onto healthy susceptible stocks, symptoms were visible 10 days after grafting. When TY172 was crossed with susceptible lines, the hybrid plants exhibited milder symptoms and lower viral content than those of the susceptible parents, yet higher than that of TY172, suggesting a partial dominance for the TY172 resistance. Upon infection of F_2 populations from these crosses, the amount of symptomless individuals appeared in a ratio approximating 7:64. This suggests that at least three genes account for the resistance.

Recently, we compared the acquisition efficiency of TYLCV by whiteflies using different infected tomato lines as source plants. Although TY172 can serve as a source of TYLCV in the field, the acquisition rate of the virus from TY172 was low compared to other resistant plant lines tested. The correlation between TYLCV accumulation level in the plant and acquisition efficiency by whiteflies will be discussed.

PROGRESS IN THE DIAGNOSIS AND EPIDEMIOLOGICAL CHARACTERISATION OF CASSAVA MOSAIC GEMINIVIRUSES IN EAST AFRICA

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Whilst cassava mosaic disease (CMD) has been recorded from East Africa for more than a century, it was not until the early 1980s that the viral aetiology of the disease was established, and only in 1994 that the first information on the Africa-wide distribution of the two cassava mosaic viruses recognised at that time as causing CMD was presented. In recent years, however, the expansion within the region of an epidemic of unusually severe CMD, apparently originating in Uganda, has encouraged renewed research effort to diagnose and characterise viruses associated with CMD. New diagnostic techniques, most notably the polymerase chain reaction (PCR), have provided greater sensitivity and capacity to discriminate between related viruses, and have revealed an increasing level of complexity. IITA and its partners in the region conducted a series of surveys between 1997 and 1998 to identify the viruses associated with CMD and assess the epidemiological implications of the single and dual infections identified. Surveys were done in Rwanda in November 1997, in Uganda in November/December 1997, and in Kenya and Tanzania in January and November 1998. Diagnoses were made with three pairs of PCR primers specific for the three cassava mosaic begomovirus forms known to occur in the region, namely, African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and the Uganda variant, EACMV-UG. ACMV occurred throughout the areas sampled, with the exception of the Kenya coast. EACMV occurred alone at the Kenya coast but together with ACMV in the important cassava-growing area along the eastern shores of Lake Victoria in western Kenya and north-western Tanzania. Dual ACMV/EACMV infections were commonly recorded in a confined region of Tanzania between the towns of Musoma and Mwanza on the eastern side of Lake Victoria. In contrast to much of Tanzania, this was a region of moderate to high CMD incidence and higher than average rates of disease spread. EACMV-UG was identified from all surveyed regions of Uganda, one location in Rwanda, parts of western Kenya and the western shoreline of north-west Tanzania (Kagera Region). Survey results showed a clear association between the distribution of EACMV-UG and the pandemic of severe CMD. In the Kagera Region of Tanzania, an 80km southward expansion of the CMD pandemic (from south-western Uganda) which occurred between the January and November surveys of 1998 was associated with a similar increase in the range of EACMV-UG. The implications of these results for cassava production in the East African region are discussed.

GEMINIVIRUSES AND CASSAVA WHITEFLIES ACROSS AFRICA

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Cassava mosaic disease (ACMD) is the most widespread and economically damaging disease of cassava in tropical Africa. A particularly virulent form of the disease has crossed Uganda from North to South and has swept into western Kenya, resulting in virtually complete yield loss in affected areas. In the 1980s the aetiology of the disease was shown to be associated with a geminivirus, by using experimental hosts and initially it was assumed that only a single virus was responsible. Recently, virus clones from ACMD have been obtained that are infectious to cassava and transmissible by whiteflies. On the basis of symptoms and the analysis of PCR products, further geminiviruses may be associated with ACMD, although conclusive evidence has yet to be published. The System-wide IPM Initiative Project, with support from DANIDA, has addressed the diversity and distribution both of geminiviruses inducing ACMD and of the potential whitefly vectors. For ACMD, PCR (using degenerate universal primers designed to amplify all dicot-infecting geminiviruses) has been used to confirm the presence of geminiviruses in infected cassava material.

Subsequent restriction mapping of PCR products, has been used to classify the identified geminiviruses into known and unidentified species. The latter were analysed to establish their relationships to the known geminiviruses inducing ACMD. In at least half the samples from Uganda more than one geminivirus contributed to ACMD. Screening adult populations of whitefly associated with cassava in the field from disparate regions of Africa has shown that more than one species is present. Genetic variability is present in both species of *Bemisia*, (*B. afer* and *B. tabaci*). High levels of polymorphism exist within *B. tabaci*, but a distinct profile appears throughout the regions sampled to date. This pattern is very similar to that observed for a biotype previously designated as "S". RAPD analysis is being used to differentiate populations of whitefly, although its use is limited when high levels of polymorphism exist. Therefore to carry out a more robust comparative genetic analysis of biotype diversity, molecular phylogenies are being constructed using DNA sequence data from the mitochondrial cytochrome oxidase I gene.

FACTORS DRIVING THE CURRENT EPIDEMIC OF SEVERE CASSAVA MOSAIC DISEASE IN EAST AFRICA

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Since the late 1980s, an epidemic of cassava mosaic disease has been spreading steadily southwards across Uganda and has it recently moved into the cassava growing areas of western Kenya and north-west Tanzania. Within the epidemic and at its leading edge, infected plants express very severe symptoms and unusually large *B. tabaci* populations are associated with rapid disease spread. Molecular diagnosis of diseased plants collected from within the epidemic revealed the presence of a new hybrid geminivirus, termed the Uganda variant.

To record both disease spread and the associated whitefly populations, eight sites were planted with healthy cassava, established at regular intervals along a transect running perpendicular to the advancing epidemic front. Adult and nymph *B. tabaci* were sampled at monthly intervals and the data showed that the epidemic's rapid spread resulted from the movement of high numbers of viruliferous, adult *B. tabaci* into previously unaffected areas.

To investigate whether vector fecundity was affected by plant-health status, three-week-old, healthy cassava plants (var. Ebwanateraka) were inoculated using either virus-free or Uganda variant-infective, pre-epidemic *B. tabaci*. Vector fecundity increased dramatically on plants infected with the Uganda variant as did the concentration of asparagine which was *c*. five times higher in diseased plants, irrespective of the presence or absence of whiteflies (in concentration diseased cassava, 0.136 $\pm$ 0.045 mg/g dry leaf weight and in healthy cassava, 0.028 $\pm$ 0.003; $P < 0.001$).

B. tabaci and the Uganda variant virus therefore share a mutually beneficial relationship that drives this epidemic and as high numbers of vectors were generated on infected cassava plants, their prompt removal from fields following symptom expression, on an area-wide basis, should generate a "virtuous cycle" of both vector and disease reduction. A rigorous phytosanitation programme that

exploits this mutualistic relationship should therefore have a major impact on a disease that currently causes estimated annual losses of US$ 1.2-2.3 bn in Africa alone.

THE ECOLOGY AND DISSEMINATION OF WHITEFLY-TRANSMITTED VIRUSES IN LATIN AMERICA

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The ecology of whitefly-transmitted geminiviruses in Latin America is primarily determined by the environmental conditions that favor the reproduction of their insect vector, *Bemisia tabaci* Genn. Two of the most important biological parameters affecting *B. tabaci* populations are temperature and relative humidity. An average temperature of 27° C and relative humidity of 70% are optimal for *B. tabaci* development. In Latin America, temperature is mainly determined by latitude and altitude, with an increasing seasonal fluctuation occurring from the Equator to approximately 35° latitude north or south In sub-tropical regions, *B. tabaci* may become economically important during the warmer months of the year, when suitable whitefly host crops are cultivated. In these regions, winter temperatures drop below the 10° C minimum level required for whitefly reproduction. The concept of vertical geography is important in Latin America because of the high elevations found in the Andean and volcanic ranges of South and Middle America, where *B. tabaci* cannot survive the relatively low temperatures in the highlands. In general terms, *B. tabaci* is a serious pest and/or efficient vector of geminiviruses at altitudes between sea level and 1,000 m. The effect of relative humidity (RH) on *B. tabaci* populations is not well understood, but it is apparent that RH values below 50% may reduce *B. tabaci* populations. Regression analyses of these environmental parameters, and geminivirus incidence in selected countries of Central America, yielded variable coefficients that only partially explain the association between these parameters and disease incidence. Further analyses on the spatial distribution of whitefly-transmitted geminiviruses in Latin America showed a high correlation between geminivirus outbreaks and drastic changes in cropping systems. These changes are closely linked to the promotion of non-traditional export crops in Latin America. Export crops often act as suitable reproductive hosts for *B. tabaci*, thus contributing to the development of large populations of the whitefly. The intensive use of pesticides in Latin America further aggravates the geminivirus problem due to the emergence of insecticide-resistant biotypes of *B. tabaci*.

GENETIC DIVERSITY AMONG GEMINIVIRUSES INFECTING CROPS AND WEEDS IN JAMAICA

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Genetic diversity of eight whitefly-transmitted geminiviruses from Jamaica was studied using non-radioactive geminivirus probes, polymerase chain reaction with degenerate primers for geminiviral DNA and nucleotide sequencing. Hybridization signals were obtained from 73% of symptomatic crops and 81% of symptomatic weeds. PCR-amplified products were obtained from 80% of plant samples which gave hybridization signals. Nucleotide identities of the common regions of DNA-A and DNA-B and partial nucleotide sequences of the 5' ends of rep, cp, bc1 and bv1 genes confirmed bipartite geminiviruses associated with *Phaseolus vugaris*, *Phaseolus lunatus* (BGMV-PR/JM), *Lyscopersicon esculentum*, *Capsicum chinense*, (tomato dwarf leaf curl geminivirus, TDLCV), *Carica papaya* (papaya mosaic geminivirus, PaMV) and two distinct geminiviruses infecting cabbage (cabbage leaf curl geminivirus from Florida, CaLCV-Fl and cabbage leaf curl geminivirus from Jamaica, (CaLCV-JM). Geminiviruses infect weeds including *Sida* spp. (sida golden mosaic geminivirus, Jamaican isolate, SidGMV-JM), *Macroptilium lathyroides* (macroptilium golden mosaic geminivirus Jamaican isolates 1 and 2, MacGMV-JM1 and MacGMV-JM2) and *Wissadula amplissima* (wissadula golden mosaic geminivirus, WGMV). Nucleotide sequence alignment of the common region and the 5' end of the rep gene were used to determine phylogenetic relationship of the Jamaican geminivirus isolates with other Western Hemisphere geminiviruses. BGMV-PR/JM was placed in the BGMV type II cluster, TDLCV, SidGMV-JM and MacMGV-JM1 were most closely related to PYMV. The geminiviruses associated with *W. amplissima* and *C. papaya* were most closely related to each other. MacGMV-JM2 was most closely related to another geminivirus infecting *M. lathyroides* from Central America. CaLCV-JM seems to be a recombinant virus between CaLCV-Fl and SidGMV-JM. These results indicate that crop-infecting and weed-infecting geminiviruses from Jamaica are distinct but phylogentically related and have several geographic origins.

ECOLOGICALLY ORIENTED MANAGEMENT STRATEGIES FOR *BEMISIA* IN AGRICULTURAL SYSTEMS

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Bemisia tabaci (Gennadius), the sweetpotato whitefly (SPW) is, historically, a tropical/subtropical insect. Its center of origin has been suggested as the Indian sub-continent. Over the last 15 to 20 years, SPW and closely related biotypes have emerged from relatively unimportant pest status to become primary pests in many areas of the world, including warm-temperate climate zones. The increasing ease of international transport has resulted in variant forms of both SPW and viruses introduced to regions where they were previously unknown. Crop losses have been huge in many areas. Increased research efforts have resulted in effective management systems. This has

been accomplished within the framework of (1) selection of non-SPW preferred cultivars, (2) spatial and temporal considerations in sequential crop systems, (3) intensive sampling and monitoring of whitefly populations, (4) chemical control focused on natural enemy conservation, established action thresholds, alternating chemistry, new chemistry, and resistance monitoring, (5) optimum crop yield goals allowing for early harvests and destruction of crop residues, and (6) active education, extension outreach to provide timely communication of new developments, SPW population dynamics, and other pertinent information to growers. These are not all-inclusive or applicable for all areas or for all crops but are general guidelines that provide the agricultural community options for consideration in SPW management.

THE MITOCHONDRIA COI GENE AS AN INFORMATIVE MOLECULAR MARKER FOR PHYLOGENETIC ANALYSIS AND IDENTIFICATION OF *BEMISIA TABACI* (GENN.), THE WHITEFLY VECTOR OF GEMINIVIRUSES

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A *Bemisia tabaci* (Genn.) species complex has been proposed to encompass all *B. tabaci*. Although this group exhibits diverse host ranges and molecular variation, and is associated with distinctive disease epidemiologies caused by the geminiviruses it transmits, it has not been possible to link distinguishing morphological features to these diverse characteristics. The genetic variation within the *B. tabaci* complex was examined by PCR-amplification and sequencing of cloned amplicons (1,100 bp) of the 3 prime two-thirds of the mitochondria COI gene (~850 bp). Sequences were used to reconstruct a phylogenetic history of *B. tabaci* and examined as a diagnostic sequence for whitefly identification using mini-BLAST analysis. The COI gene marker was assessed for select field-collected and colony-maintained reference *B. tabaci* and for several different whitefly species and genera, as outgroups. Parsimony and distance analyses resolved several non-*B. tabaci* outgroup taxa and one main *B. tabaci* group that contained within it multiple Old World clades, with a basis in their respective region or host plant of origin, and one clade that contained all New World *B. tabaci*. Old World *B. tabaci* exhibited far greater divergence from one another than did New World taxa. In the absence of useful morphological characters for identification of *B. tabaci* and other Aleyrodidae, a COI sequence database is under construction to facilitate whitefly identification by comparative sequence analysis.

THE CORE REGION OF THE COAT PROTEIN GENE OF VIRUSES OF THE *GEMINIVIRIDAE* IS PHYLOGENETICALLY INFORMATIVE: INTRODUCING AN INTERACTIVE WEBSITE FOR *BEGOMOVIRUS* IDENTIFICATION USING THE CORE CP SEQUENCE

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Begomoviruses are globally emergent pathogens but it is not yet possible to achieve accurate and rapid identification of these viruses. Several prospective molecular markers in the begomovirus genome were assessed to identify viral sequences that were useful for reconstructing phylogenetic histories and for achieving virus identification by PCR amplification and sequencing of the specified target region. Sequences of the complete viral genome/A component, the coat protein gene (*AV1*), the 5' 200 nucleotides of *AV1*, and the core region of *AV1* (core *CP*) were evaluated. Criteria considered optimal were: conserved regions conducive to primer design for the exclusive detection of begomoviruses, an amplicon suitable in size to sequence in a single run, and a phylogenetically informative region. Results indicated that the core *Cp* marker had all these desirable attributes and was useful for achieving accurate virus identification or for obtaining the closest possible match to an extant geminivirus quasispecies. An interactive data base containing globally representative begomovirus core *Cp* sequences has been constructed and is now available on the server at the NSF-IPM Center, Raleigh, NC. This collaborative effort involves Dr. R. Stinner, IPM Center, North Carolina State University, and the virology laboratories in the Department of Plant Sciences at the University of Arizona and in the Department of Plant Pathology, Washington State University. At this site, the core *Cp* sequence from an unidentified field isolate can be entered to achieve identification or a closest match (% sequence identity) by mini-BLAST comparison to a panel of reference sequences placed at an interactive Website Online: http://www.ipmnet.org/GEMINI/. Each discrete viral species is linked to a separate page at the site that contains additional information on the global distribution, host range, symptoms, and other relevant information about each well-characterized virus. This approach permits for the first time the accurate identification of begomoviruses and is somewhat analogous to serological approaches involving antibody production to the viral coat protein. For the first time, the molecular tracking of whitefly-transmitted geminiviruses is possible globally, and such efforts will be instrumental in the early detection of exotic introductions and emerging viral genotypes.

POSTER PRESENTATIONS

VARIATION IN HOST RESPONSE TO *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE) IN TOMATO PLANTS

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Greenhouse (20°C; Tmax. = 28°C; Tmin. = 14°C; 65-70% R.H.) and field (17.5ºC to 36.5ºC) no-choice assays were performed to investigate the settling behavior of *Bemisia argentifolii* Bellows & Perring (equivalent to the Spanish B-biotype of *B. tabaci* Gennadius) on several tomato plants with different acylsugar contents. Plants tested were *Lycopersicon esculentum* Mill. cultivars ALTA and PETO 95, wild species *L. pennellii* (Corr.) D´Arcy LA716 and tomato lines 94GH-006 and 94GH-033 (F_3 backcrosses from PETO 95 with LA716). The best reproductive activity in terms of fecundity and fertility was observed on ALTA (total acylsugar content = 0 µg/cm^2) and it was null on LA 716 (total acylsugar content = 37.75 ± 2.82 µg/cm^2), but a clear relationship between low acylsugar levels in the other plants tested and whitefly reproductive parameters was not found.

Comentario [1]:

The host response to the Spanish B-biotype of *B. tabaci* of *L. esculentum* Motelle, VFN8 and Ronita [varieties carrying the *Mi* gene, which confers resistance to root-knot nematodes *Meloidogyne* spp. and to the potato aphid *Macrosiphum euphorbiae* (Thomas)], was compared with that of Moneymaker, Río Fuego and Roma (tomato cultivars lacking-*Mi*) in a free choice experiment under greenhouse conditions [(23ºC:15ºC (day:night) and 70-80% R.H)]. When the six cultivars were considered separately, whitefly infestation and reproduction were lower on tomato plants bearing *Mi* than on the other varieties. When all *Mi*-bearing plants were pooled together, the differences compared with plants lacking *Mi* were statistically significant ($p<0.05$) for all the parameters considered.

Because tomato resistance to *B. tabaci* was not clearly correlated with the total acylsugar content under a threshold level and a differential host response in tomato plants with or without *Mi* gene was observed, it is postulated that this gene can be involved in alternative mechanisms of resistance to this insect, not associated with these leaf trichome exudates. Further studies will seek to demonstrate whether *Mi* is finally the main gene responsible of this resistance and whether similarities exist among the resistance mechanisms to whiteflies, aphids and nematodes, which could be of interest in commercial tomato breeding programs.

SETTLING BEHAVIOR OF *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE ON SOME COMMON WEEDS IN SPAIN

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Five Spanish winter weeds and five summer weeds were screened for host preference of *Bemisia tabaci* (Gennadius). In a laboratory no-choice assay at 20 ºC:15 ºC, 14h photoperiod and 65:70% relative humidity, fecundity and fertility of the silverleaf whitefly *Bemisia argentifolii* Bellows & Perring, corresponding to the Spanish B-biotype of *Bemisia tabaci* (Gennadius), were influenced by winter weeds. The greatest number of eggs, pupae and adults were obtained with cheesweed, *Malva parviflora* L. (25, 21 and 19, respectively) followed by shepherds purse, *Capsella bursa-pastoris* L. (16, 12 and 10), wild mustard, *Brassica kaber* (DC) (12, 5 and 4), and prickly lettuce, *Lactuca serriola* L.(6, 3 and 2), meanwhile the oviposition was nil on common fiddleneck, *Amsinckia intermedia* F. & M. The percentage of adult emergence (from egg to adult) was significantly higher ($p<0.001$) when *M. parviflora*, *C. bursa-pastoris* and *B. kaber* were the hosts (76, 64 and 35, respectively) compared with *L. serriola* (26).

In a greenhouse choice experiment with summer weeds at 23ºC:19ºC (day:night) and 70-80% R.H, adults of the Spanish B and Q-biotypes of *B. tabaci* preferred *Datura stramonium* L. and *Solanum nigrum* L. to *Amaranthus retroflexus* L., *Chenopodium album* L. and *Echinochloa crus-galli* L. For both, B and Q biotypes, significantly higher daily infestation of whiteflies and a larger number of pupae per plant (including empty pupal cases) were observed in *S. nigrum* than were in *D. stramonium.* However, the number of pupae and empty pupal cases per leaf was significantly greater on *D. stramonium* because of the higher number of *S. nigrum* leaves. Adult abundance of B and Q-biotypes on *E. crus-galli* was nil and no males or females of Q-biotype were observed on either *A. retroflexus* or *C. album.* The infestation of B-biotype adults was very low in *A. retroflexus* and *C. album.* Development from egg to adult on *D. stramonium* and *S. nigrum* was faster for B-biotype (22 days) than for Q-biotype (28 days).

From these results it was concluded that control of *M. parviflora*, *B. kaber*, *C. bursa-pastoris*, *D. stramonium* and *S. nigrum* is recommended to avoid the infestation risk of *B. tabaci* on horticultural crops where the presence of whiteflies is a major problem.

IDENTIFICATION OF SOME COMMON WEEDS AS RESERVOIRS FOR TOMATO YELLOW LEAF CURL VIRUS TRANSMITTED BY *BEMISIA TABACI* (GENNADIUS)

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Weeds have been reported to be important reservoirs of whitefly-transmitted viruses and play a significant role in the epidemiology of some virus species. Tomato yellow leaf curl virus (TYLCV), a geminivirus transmitted by *Bemisia tabaci* (Gennadius) causes devastating disease in many tropical and subtropical regions, particularly in the Mediterranean Basin. In order to identify the role of different weed hosts of *B. tabaci* in TYLCV epidemics, 12 replicates of each of two summer weeds (*Datura stramonium* L. and *Solanum nigrum*, L.) and three winter weeds [(*Brassica kaber* (DC), *Capsella bursa-pastoris* (L.) Medic. and *Malva parviflora* L.)] were tested in a laboratory assay for TYLCV transmission by the Spanish B-biotype of *B. tabaci*. Whiteflies were placed on TYLCV-M (Murcia, Spain)-infected source plants [*Lycopersicon esculentum* (var. Río Fuego)] for a 72-h acquisition access period. Fifteen viruliferous whiteflies per plant were then transferred to each of the test weed plants. After a 72-h inoculation access period whiteflies were transferred back to healthy tomato test plants. Four weeks later, both tomato and weed plants were checked for TYLCV symptoms and the infection was confirmed by ELISA and PCR tests.

B. tabaci transmitted TYLCV from infected tomato plants to *S. nigrum and D. stramonium* and *vice versa*. A high proportion of infected plants was obtained in *S. nigrum* (7/12) and *D. stramonium* (10/12). Neither of the other weed species was infected by TYLCV. Although *B. kaber*, *C. bursa-pastoris* and *M. parviflora* are reported as suitable hosts of *B. argentifolii* Bellows & Perring (equivalent to the Spanish B-biotype of *B. tabaci*), and *M. parviflora* has also been reported as a host of TYLCV, this biotype of *B. tabaci* could not transmit TYLCV to these weeds in our experiments. Our study has shown that *S. nigrum and D. stramonium* could be TYLCV reservoirs and may play an important role in epidemics of TYLCV.

LABORATORY EVIDENCE OF INTERBREEDING BETWEEN BIOTYPES OF *BEMISIA TABACI* (HOMOPTERA, ALEYRODIDAE) PRESENT IN SPAIN

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Two characterized biotypes of *Bemisia tabaci* (Gennadius) present in Spain, biotypes B (= *Bemisia argentifolii*) and Q were studied in order to determine whether they interbreed. Normally, according to the literature, inter-breeding between different biotypes of *B. tabaci* has not been considered possible, as shown in several experimental assays. Only recently, a study has showed laboratory and field evidence for inter-breeding between biotypes of *Bemisia tabaci* in Australia: the B type and two non-B types.

Two Spanish populations belonging to B and Q biotypes and reared in our laboratory for several generations were used in this study. All possible crosses among the two biotypes were performed, in two kinds of experiments: **1.** The analysis of the sex-ratio in the eggs produced by mature females (14 days-old) after two days of mating and oviposition, and when five couples were placed together. **2.** The analysis of the sex-ratio in the overall offspring produced by a female, when single pairs were confined and with continuous presence of a male (dead males were replaced).

Due to the haplo-diploidy in *B. tabaci*, males are produced from unfertilized eggs and females from fertilized eggs; therefore, the presence of F1 females indicates crossing between adults. In

addition, to confirm this point, parents and offspring were analyzed with molecular markers for B and Q biotypes, using the RAPD-PCR technique.

Results showed the presence of females in some of the inter-population crosses and for the two kinds of experiments, although the number of females produced was much smaller than in the intrapopulation crosses, which showed that inter-breeding between B and Q biotypes was possible but it would be hard to produce. Moreover, in the F1 generation, "hybrid" females showed amplification bands for both biotypes, but males had typical bands for the "mother" biotype.

Inter-breeding in the laboratory and field merits further study in relation to the *B. tabaci* pest problem.

GENETIC RELATIONSHIP OF BIOTYPES OF *BEMISIA TABACI* (HOMOPTERA:ALEYRODIDAE) PRESENT IN SPAIN BASED ON RAPDS AND AFLPS

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The existence of biotypes of *Bemisia tabaci* Gennadius is a fact widely documented. The identification of the biotypes present in a zone is relevant for the epidemiology of the viruses they transmit, given that the biotypes can have different vector efficiencies. Three biotypes have been reported in Spain to date. The B biotype, detected in Canary Islands, Málaga, Almeria, Madrid and Barcelona, occurs worldwide. The Q biotype has been observed in Seville, Málaga, Almeria, Murcia, Valencia and Majorca Island. This biotype has been found also in Portugal and Tunisia. Finally, the biotype S has been located in a single locality on a plant of *Ipomoea* in Nerja (Málaga).

Two different studies were carried out in order to characterize the inter- and intra-populational genetic variation of several Spanish populations. First, their relationship with other biotypes and populations from the world was determined by using the AFLP technique. The highest similarity of biotype Q was with a population from Nigeria (0.59), and both were the most closely related to biotype B. Biotype S was similar (0.75) to a different population from Nigeria, and both were least similar (0.27) to other biotypes of *B. tabaci*.

In a second experiment, 56 individuals of 6 populations were studied by RAPD-PCR. The 336 individuals were completely discriminated by means of 234 scored bands. Average substitutions per nucleotide site within populations, oscillated from 0.0177 from a population of biotype B from Tenerife Island to 0.0392 of a population from Almeria composed of a mixture of B and Q biotypes. No hybrids were observed in this mixed population, suggesting that there is no inter-breeding in the field conditions of the area.

WEEDS AS RESERVOIRS OF TOMATO YELLOW LEAF CURL VIRUSES IN SPAIN

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Since first reported in 1992, tomato yellow leaf curl virus (TYLCV) has become a major limiting factor for tomato (*Lycopersicon esculentum* Mill.) production in south and south-eastern Spain. In addition, it has recently been reported in common bean (*Phaseolus vulgaris* L.) causing the disease named bean leaf crumple. Isolates corresponding to both TYLCV-Sr and TYLCV-Is viral species have been reported to be involved in epidemic outbreaks. Both viruses are transmitted naturally by the whitefly (Homoptera: Aleyrodidae) *Bemisia tabaci* Genn. in a circulative persistent manner and they have narrow experimental host ranges.

Development of feasible control procedures against TYLCV has been difficult because of the emergence of *B. tabaci* populations resistant to commonly used chemicals and the lack of commercial cultivars resistant to the virus. Consequently, crop management practices play a critical role for disease control. In the TYLCV pathosystem, weeds could be important in the epidemiology of TYLCV as reservoirs of primary inoculum. A knowledge of the natural reservoirs of TYLCV in a certain area will help in the development of effective strategies to manage viral epidemics. Thus, this study was undertaken to identify and establish the importance of weed species as reservoirs in TYLCV epidemics in Spain. Surveys were carried out during 1996, 1997 and 1998 throughout the main tomato growing regions of south and south-eastern Spain affected by TYLCV epidemics. Samples were collected of the most frequent weed species growing in the vicinity of tomato crops. In addition to random surveys, samples from weeds with virus-like symptoms were also collected. More than 1,200 plants representing 52 species in 18 families were collected and analysed for TYLCV-Sr and -Is infection by molecular hybridisation of prints of petiole cross sections on nylon^{+} membranes, using digoxigenin-labeled DNA probes specific to each virus species. Positive results were confirmed by polymerase chain reaction amplification of DNA and by Southern blot hybridisation of total nucleic acids. Results showed that these viruses are not widespread in weed populations. *Solanum nigrum* L. was the only natural reservoir found for TYLCV-Sr. TYLCV-Is was detected in *Datura stramonium* L., *Mercurialis ambigua* L. fil., and *Ipomoea indica* (J. Burman) Merrill. This is the first report of *M. ambigua* and *I. indica* as hosts of TYLCV-Is.

THE USE OF NETS AND INSECTICIDE TREATMENTS IN THE CONTROL ON *BEMISIA TABACI* GENN. POPULATIONS AND TOMATO YELLOW LEAF CURL VIRUS SPREAD IN PROTECTED TOMATO CROPS IN PORTUGAL

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Tomato yellow leaf curl virus (TYLCV) is a whitefly-transmitted geminivirus responsible for an economically important disease in tomato (*Lycopersicon esculentum* Mill.) crops in many tropical, sub-tropical and temperate regions of the world. In 1995, TYLCV was first reported in Algarve (southern Portugal), associated with a severe tomato disease. The TYLCV vector, *Bemisia tabaci* Genn., occurs throughout the whole year in Algarve region, where TYLCV epidemics have occurred annually in greenhouse tomato crops and quite often the disease incidence is up to 100%. In spring 1998, TYLCV was also found associated with a damaging disease of green bean (*Phaseolus vulgaris* L.).

Field trials are being carried out in order to study the epidemiology of the TYLCV and to establish control strategies for the sustainable management of the TYLCV/ *B. tabaci* complex in Portuguese cropping systems. A field trial was performed to evaluate the effect of the use of screen nets and insecticide sprays both on *B. tabaci* populations and TYLCV spread. The trial using the tomato cv Daniela was set up in the beginning of September 1997 and included four plots: I - with nets and without insecticides; II - with nets and insecticide; III - without nets and with insecticidess and IV - without nets and insecticides. The reference to the plots with or without insecticides deals only with the control of *B. tabaci*. The trial finished by the end of March 1998. The whitefly population was monitored weekly by yellow sticky traps and visual observation of larvae and adults on 25 plants/plot. In the whole trial all the plants were observed weekly in order to record TYLCV symptoms. The number of *B. tabaci* adults was high, during September and October, in the plots without nets and it was low in the other two plots. The first symptoms of TYLCV were observed about 3 weeks after plantating and the effect of using nets was clear 6 weeks after planting. The final percentages of infected plants in the plots without nets were, respectively 70% (III) and 100% (IV), and 16% (I) and 17% (II) in the other plots. Despite increasing numbers of *B. tabaci* adults in plot I after the end of October, the final percentage of symptomatic plants was low. The insecticide spray program used (imidacloprid alternated with endosulfan) maintained a low population of the vector, nevertheless it did not prevent the virus spreading.

These preliminary results will be discussed and complemented with the ones from the current second-year trial, as well as with the on-going trial to assess the role of trap crops on TYLCV incidence.

THE EFFECT OF VARIETAL MIXTURES ON THE PROGRESS OF CASSAVA MOSAIC VIRUS DISEASE GROWN UNDER EPIDEMIC CONDITIONS IN UGANDA

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An experiment was conducted using four cassava varieties namely Ebwanateraka (susceptible/local), Nase 2 (moderately resistant/improved), SS4 and Migyera (resistant/ improved) to assess the progress of CMD in each variety alone and when grown as a mixture. There were significant differences in CMD incidence and in the areas under the disease progress curves amongst varieties alone and in the mixture in the 1995/96 and 1996/97 experiments. Ebwanateraka had the highest incidence and SS4 the lowest. Cultivation as a mixture not only significantly reduced overall incidence to levels comperable to the resistant Nase 2 but also decreased the whitefly vector population. Compared to single variety stands, incidence of CMD was reduced significantly only in Ebwanateraka while vector populations were less only in SS4 and Nase 2 when planted in mixtures, although single variety plots were always associated with higher incidence and vector population than when mixed. In varietal mixtures, the expected incidence of CMD and population of adult whiteflies based on the results for the varieties when grown alone were consistently and significantly lower than actual values for much of the two seasons. There was no statistically adquate relationship between the incidence of CMD and the population of adult whiteflies recorded the previous month for any of the varieties in either experiment. However, statistically strong relationships were established between adult whitefly populations and leaf area index (LAI). The implications of the findings in the epidemiology and management of CMD and the scope for future research are discussed.

AN OVERVIEW OF THE INCIDENCE OF CASSAVA MOSAIC DISEASE IN EAST AFRICA, 1998 UPDATE

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A study conducted in Uganda and the lake Victoria region of Kenya and Tanzania towards the end of 1997 revealed that the status of cassava production in East Africa is deteriorating. This has been most apparent in Uganda where, between the late 1980s and the present day, much of the country's cassava production has been devastated by an epidemic causing unusually severe

symptoms. The data collected in the recently concluded survey indicates that CMD incidence was highest in Uganda (67%) followed by western Kenya (51%) and Tanzania (24%). In Uganda, disease incidence was highest in the central region (80%) and lowest in the south western region (47%), where most of the diseased plants had been infected recently by whitefly (38%). Disease incidence was highest in the Western province (85%) of Kenya, bordering Eastern Uganda and lowest in the Nyanza province (13%) towards the Tanzania border. CMD incidence was generally low in Tanzania. The highest incidence was recorded in the Coastal region (40%) an area stretching from Dar es Salaam to Tanga, while Mtwara region in the south of the country had the lowest (13%) disease incidence. The results clearly indicate the use of CMD-infected cutting material as an important means of virus dissemination. However, the mainly whitefly-borne CMD incidence in the southern districts of Rakai and Masaka in Uganda towards the Tanzania border poses a serious threat to north-western districts of Tanzania, bordering Uganda.

THE GEOGRAPHIC DISTRIBUTION OF CASSAVA *BEMISIA TABACI* BIOTYPES IN RELATION TO THE CURRENT EPIDEMIC OF CASSAVA MOSAIC DISEASE IN EAST AFRICA

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The cassava whitefly, *Bemisia tabaci*, in West Africa is isolated reproductively from the sympatrically occurring okra biotype, which has a different and wider host-plant range. The cassava biotype is the vector of cassava mosaic viruses (Family: *Geminiviridae*: Genus, *Begomovirus*), which cause cassava mosaic disease (CMD). In East Africa during the last decade, an epidemic of severe CMD has been progressing steadily southwards across Uganda and has recently crossed into western Kenya and north-western Tanzania. This CMD epidemic is associated with unusually high numbers of *B. tabaci* and one possible explanation for this is that a 'better adapted', more fecund, biotype is expanding its range southwards and, at the same time, causing an increased transmission rate of severe CMD.

To investigate whether the *B. tabaci* population associated with this epidemic is isolated reproductively from that found ahead of it, reciprocal crosses were set up between combinations of ten *B. tabaci* cultures collected from the two zones. Unmated female *B. tabaci* produce male offspring only and therefore the presence of female offspring in all the crosses indicated that there is no mating barrier between epidemic and pre-epidemic Ugandan *B. tabaci*. The offspring of these crosses were self-crossed to produce an F_2 generation and all produced both male and female offspring. The control crosses between cassava and cotton *B. tabaci*, carried out on the common host egg-plant, produced only male offspring. The whitefly populations and the offspring resulting from the crosses were characterised molecularly by RAPD-PCR, which confirmed the above results.

To investigate further whether more than one reproductively isolated cassava *B. tabaci* biotype exists, reciprocal crosses will also be conducted between cultures originating from south India, Uganda and Tanzania and the results will be discussed.

INTEGRATING VARIOUS NEONICOTINOID INSECTICIDES INTO CHEMICAL CONTROL PRACTICES FOR SUSTAINABLE WHITEFLY MANAGEMENT

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The neonicotinoids represent one of the premier new classes of insecticides and have proven to be highly effective against aphids and whiteflies. Their timely introduction, beginning with the compound imidacloprid, has helped quell chronic whitefly outbreaks such as those that occurred in the Imperial valley, CA during the early 1990s. The compounds acetamiprid and thiamethoxam also demonstrate superb activity against homopterans and help to diversify the insecticides available by providing excellent control when used as foliar sprays. Each of these compounds acts upon the nicotinic acetylcholine receptor of insects, a target that is not utilized by pyrethroids, organophosphates or carbamates. Therefore, populations of whiteflies and aphids showing high levels of resistance to conventional insecticides have proven highly susceptible to the neonicotinoids. Concerns over potential resistance development to one or more compounds in this group have been expressed in the light of the heavy reliance placed upon imidacloprid in regions such as Almería (Spain) and Imperial Valley. The multiple uses of imidacloprid throughout annual cropping cycles coupled with long persistence in treated plants, especially so for systemic treatments of imidacloprid, have placed this particular compound and perhaps subsequent neonicotinoids at a seemingly high risk for resistance development. Thus, there is an urgent need for implementing resistance management strategies to prolong efficacies of the neonicotinoids. Various strategies will be discussed with respect to integrating the neonicotinoids into a diversified program of chemical control to avoid high selection pressure on any one compound or class of insecticides. Implementation of any chemical management strategy must be understood in the context of a wider integrated management strategy that maximizes efforts to incorporate non-chemical controls for suppression of aphid and whitefly populations. However, the explosive capacity of homopterans in optimal environments at times mandates heavy reliance upon insecticides, and they must therefore be used in the most judicious manner to avoid the pitfalls of resistance.

SESSION 4

MODELLING PLANT VIRUS EPIDEMICS

ORAL PRESENTATIONS

A THEORETICAL ASSESSMENT OF VECTOR-VIRUS TRANSMISSION MECHANISMS ON PLANT VIRUS DISEASE EPIDEMICS

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There are four general transmission classes of plant viruses transmitted by Homopteran insects, 1) non-persistent, 2) semi-persistent, 3) persistent and circulative, and 4) persistent and propagative. These are characterized by rate of acquisition of the virus by the insect from the host plant, rate of inoculation of host plants by infectious insects, and length of the latent period in the vector. The influence of these three factors on virus disease dynamics and on management strategies was explored using the linked-differential-equation model of the host and vector populations developed by Jeger, van den Bosch, Madden and Holt (*IMA Journal of Mathematics Applied in Medicine and Biology* [1998] 15: 1-18). During an epidemic, four categories of plant-host disease status are considered, namely healthy (disease-free; *H*), latent (*L*), infectious (*S*), and removed (*R*); diseased plants move through the categories at rates specified in the model. Three categories of vector status are considered, namely virus-free (*X*), latent (*Y*), and infective (inoculative; *Z*), although *Y* may be, by definition, zero for non-persistent and semi-persistent classes. New diseased plants occur in the model through the product of *H*, *Z*, and a contact rate, with the rate being a function of number of plants visited by a vector, feeding time per visit, and mean time that a vector must feed to inoculate a plant. The consequences of these transmission classes can be explored theoretically by determining equilibrium values of the plant and vector categories as a function of model parameters, as well as with thresholds for the increase in disease. Numerical results can also be obtained under a wider range of conditions. The model clearly shows the difference between the persistent-propagative class and the other transmission classes, both in terms of rates of disease increase and effects of control measures. Applications of the model will be presented.

A NEW GENERAL MODEL OF PLANT-VIRUS DISEASE INFECTION WHICH INCORPORATES VECTOR AGGREGATION

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In plant virus epidemiology, dynamical models have invariably incorporated an infection (inoculation) rate which is of a bilinear form being directly proportional to both the abundance of healthy (susceptible) hosts, and to the abundance of infective vectors. Similarly the acquisition rate is usually directly proportional to the abundance of non-viruliferous vectors, and that of infectious hosts. These assumptions have been questioned for certain human diseases and infection rates which

incorporate power parameters of the variables have been proposed. Here we examine infection rates for plant virus diseases which are of a more general form than the familiar bilinear terms. For plant-virus diseases, the power parameter can be regarded as a measure of the spatial aggregation of the virus vectors or as a coefficient of interference between them, depending on the context.

We examined field data for cassava mosaic virus disease (CMD) incidence and found that disease progress curves and vector abundance curves over the first 6 months of the crop could not be explained using bilinear infection rates. Incorporating the new infection rates allowed the range of observed curve types to be described.

New evidence of a mutualistic interaction between CMD viruses and vectors has shown that spatial aggregation of the vectors is an inevitable consequence of infection, particularly with a severe virus strain or a susceptible host. In the models, vector aggregation reduces the effective contact rate and therefore the predicted equilibrium abundance of infected hosts is rather less than when a bilinear contact rate is used. Two forces are at play in the mutualistic interaction: virus infection increases vector fecundity, but aggregation of the vectors may mean that disease spread *within that crop* is less than would be expected by such a boost in vector number.

The large number of vectors generated on infected plants emigrate to alight on other crops in large numbers and so promulgate continued disease spread. Using a spatially-structured version of the model, the effect of the mutualistic interaction between virus and vector on the propagation of the epidemic wave of CMD seen recently in Uganda, was examined.

MODELLING PLANT VIRUS EPIDEMICS IN A COMBINED FIELD-NURSERY SYSTEM

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During the last few years a number of papers have appeared on modelling plant virus epidemics. Chan & Jeger (1994) introduced a model describing plant-virus dynamics with roguing and replanting and derived invasion criteria for viruses into disease-free crops. Holt *et al.* (1997) explicitly incorporated vector dynamics into a model of cassava mosaic disease and studied the dynamics of the disease on a regional scale as in the current epidemic in Uganda. Jeger *et al.* (1998) compared virus transmission characteristics and incorporated vector dynamics, activity and migration into a general epidemic model.

For some perennial crops the material used for replanting is derived from cuttings taken from the crop, which are then multiplied in a nursery. We modelled this situation to determine effects on plant virus epidemics and whether dynamics were influenced more by roguing in the field or the nursery. The plant populations were divided into healthy and diseased categories and were linked according to a basic SI model of disease transmission. Roguing and replanting in both the field and nursery were included. Two variants of the model were developed in which plants from which cuttings were taken either remained in the field or were harvested.

A criterion was derived for the invasion of diseased plants into the healthy field crop, and consisted of three terms: the basic reproductive numbers of disease in the field and in the nursery,

and the cycling of diseased material between the field and nursery. Disease can still invade when the basic reproductive numbers are less than 1 depending on the magnitude of cycling. Under some conditions only diseased plants remain in the field. An invasion criterion was also derived for healthy plants and depended on replanting rates in the field and nursery, and removal rates of healthy plants caused by infection, roguing and mortality. A graphical analysis of the invasion criteria and a sensitivity analysis suggest that roguing in the field is generally more effective in the control of a virus disease than roguing in the nursery.

THE INFLUENCE OF CULTIVATION PRACTICES ON THE SPATIAL DISTRIBUTION OF VIRUSES IN AUSTRALIAN HOP GARDENS

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In Australia, hops are frequently infected by hop latent virus (HpLV), hop mosaic virus (HpMV) and Prunus necrotic ringspot virus, apple (PNRSV-A) and intermediate (PNRSV-I) serotypes. In the absence of the most efficient aphid vector, *Phorodon humuli*, the spread of HpMV and HpLV relies upon the activity of other aphid species and/or mechanical transmission. Pollen-associated transmission of PNRSV in hop is not important, and while mechanical transmission and below-ground root contact have been implicated, the spread mechanism of PNRSV in hop is unclear. This study examined whether cultivation practices influence spatial distribution of viruses in Australian hop gardens.

Two plots of cv.Victoria (established virus-free) were surveyed for virus incidence. Plot 1 (n=1275) was planted in 1989 at Bushy Park, Australia, where cultivation practices operate only along rows. Plot 2 (n=500) was planted in 1994 at Myrtleford, Australia. Here cultural practices operate across and along rows. In 1990 individual plants from plot 1 were tested by ELISA for HpMV and PNRSV (A&I). In 1996 and 1997 plants were also tested for HpLV. Individual plants from plot 2 were tested in 1998 for all viruses. Epidemics were characterised spatially by ordinary run analysis (ORA) and radial correlation analysis (RCA), the latter by 2DCORR.

In plot 1, (1996 and 1997) ORA detected significant clustering of all three viruses in the same direction as cultivation practices. Epidemics of all three viruses were too anisotropic within rows for RCA to conclude significant spatial correlation. In plot 2, results from ORA and RCA, indicated a random distribution of HpLV and HpMV, and aggregation in both directions of PNRSV. These results imply the role of cultivation practices in spread of PNRSV. The autocorrelated incidence of HpLV and HpMV in plot 1 may result from secondary spread by mechanical transmission or vectors moving to adjacent plants. At site 2 random incidence suggests the spread of HpLV and HpMV was more reliant upon primary infection with little secondary spread.

REGIONAL ANALYSIS OF PLANT VIRUS EPIDEMICS WITH GEOGRAPHIC INFORMATION SYSTEMS AND GEOSTATISTICS

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Virus disease management is most consistent if there is a relatively simple relationship between the virus source and the crop. Examples include viruses with a single host or viruses whose primary dispersal mechanism is by seed transmission or vegetative propagules and certification programs are effective. Management of ecologically complex virus diseases, best typified by whitefly-transmitted geminiviruses, can be improved by the use of more sophisticated information processing technology such as geographic information systems (GIS) and geostatistics to guide cultural management decisions. A focus on suppressing virus and vector sources is the cultural tactic most likely to be successful in reducing virus damage.

A GIS is a computer system capable of assembling, storing, manipulating, and displaying data referenced by geographic coordinates. GPS receivers determine location using a system of navigation satellites operated by the United States Department of Defense (the NAVSTAR system) and are among the most important tools for spatially referencing agriculture data. GIS relates the data collected by GPS to other sources of geo-referenced information. Regional applications in plant virus management have used inexpensive hand-held GPS units, because the interpretation of spatial patterns spanning over 100 km does not require an accuracy of better than 100 m for point coordinates.

Geostatistics is a set of tools for interpolation to create surface maps based on point samples or observations. A surface map is a map with an area shaded in a color or gray scale keyed to a variable. Surface maps allow the viewer to grasp the larger picture without being distracted by the scatter of point data. Regional surface maps are appropriate when a variable is correlated with itself at various separation distances and directions beyond field boundaries. This is particularly true in plant virus disease situations where nearby fields are frequently exposed to similar pressure from alternative hosts of the virus embedded in the landscape. Recurring patterns of incidence and risk of virus disease at a regional scale can develop because of the cumulative effects of local landscape elements. GIS and geostatistics help in understanding and communicating these site-specific patterns.

USE OF A MATHEMATICAL MODEL AS AN ANALYTICAL TOOL TO PRIORITIZE IPM RESEARCH AND INTERVENTIONS FOR WHITEFLY-TRANSMITTED GEMINIVIRUSES IN LATIN AMERICA

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Geminiviruses transmitted by the whitefly *Bemisia tabaci* (Gennadius) have caused major epidemics and yield losses in horticultural crops in Latin America. Tomato (*Lycopersicon esculentum* Mill.) has been one of the most severely affected crops. Reviewing the available literature on IPM options to protect tomatoes from whitefly-transmitted geminiviruses, many IPM tactics exist, including resistant varieties, synthetic and natural insecticides, cultural practices, physical barriers, biological and legal controls. With a growing variety of IPM options, researchers and producers are faced with the need to recommend and select viable IPM tactics, often, without previous field testing and implementation. Recently, a mathematical simulation model for insect-transmitted viruses has been developed and applied to analyze the *Bemisia*-transmitted geminivirus pathosystems. This presentation focuses on the specific cases of tomato-infecting geminiviruses in Latin America, and tomato yellow leaf curl begomovirus (TYLCV), an Old World geminivirus recently introduced into the Caribbean from Israel. As a result of the analysis, recommendations are made on the epidemiological research needed, and the most epidemiologically-effective IPM tactics to be prioritized for further development.

EPIDEMIOLOGY OF TWO PHYTOPLASM STRAINS ASSOCIATED WITH YELLOW CRINKLE IN PAPAYA

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Phytoplasmas, previously called mycoplasma-like organisms or MLOs, have been reported in Australia since 1902 when big bud disease of tomato was first described. Differentiation studies have found that two main groups of phytoplasmas are associated with these diseases: the faba bean phyllody strain cluster which includes the tomato big bud (TBB), and the stolbur group of phytoplasmas which includes papaya dieback (PDB). Another variant of the TBB group is a phytoplasma called V4, which was first transmitted by dodder from a sweet potato with little leaf to periwinkle (Vinca), and has since been found to occur in other field-collected plant hosts.

Phytoplasmas have been shown to be associated with three diseases of papaya (*Carica papaya*) in Australia: mosaic, yellow crinkle, and dieback. In 1996, papaya trees at a plantation situated in Katherine, *c*. 300 km south of Darwin were observed with yellow crinkle disease and phytoplasma was found to be associated with this disease. Subsequently, we investigated phytoplasma variability within this plantation to determine the types of phytoplasmas.

The temporal and spatial spread of two phytoplasma strains (TBB and V4) that cause yellow crinkle disease of papaya were monitored in a papaya plantation (65 rows x 55 plants/row) located near Katherine, Northern Territory, Australia. Beginning in May 1996, each plant was inspected

monthly and the location (row, plant number) and date of each symptomatic plant was recorded. Leaves from symptomatic plants were sampled and stored at 4^{o} C until processed. Phytoplasmas were distinguished by restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA PCR product. Restriction enzymes used were Alu and Rsa I and digested products were resolved on 8% polyacrylamide gels.

Through September of 1998, TBB and V4 occurred in nearly equal frequencies with the incidence of V4 and TBB-diseased plants reaching 10.5% and 9.0%, respectively. Time between symptom appearance and plant death ranged from 1 to 10 months in plants infected by the TBB strain and from 1 to 8 months for plants infected by the V4 strain. The mean time from symptom detection to plant death was 3.5 months for plants infected by the V4 strain and 4.0 months for plants infected with the TBB strain. As of September 1998, the temporal rate of disease spread (based on symptoms) for the V4 and TBB strains was 0.19 (R^2 = 0.90) and 0.16 (R^2 = 0.97) units/month, respectively. The rate of plant death caused by either TBB and V4 was *c*. 0.15 units/month (R^2 = 0.98). Between May 1996 and September 1998, the doubling time for plant death decreased with time, indicating that the yellow crinkle epidemic is currently in the exponential phase of growth. Time to 50 % plant death is predicted to occur *c*. 16 to 19 months after September 1998.

FORECASTING APHID OUTBREAKS AND SPREAD OF CUCUMBER MOSAIC VIRUS IN *LUPINUS*: A SIMULATION MODEL FOR A MEDITERRANEAN-TYPE CLIMATE

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Cucumber mosaic virus (CMV) causes a serious disease of narrow-leafed lupin (*Lupinus angustifolius)*. It is seed-borne in lupins and seed-infected plants act as the primary virus source for secondary spread by aphid vectors in crops. It causes yield losses of up to 60% in some years, but has little impact in others. Aphids also cause sporadic yield losses in lupins due to direct feeding damage. The spread of CMV depends on a number of variables: most importantly the magnitude of the initial CMV infection source (seed-infected plants), plant density, available ground-cover, the time of vector arrival in relation to plant growth stage, how many vectors acquire virus, how often they move to a new plant, their transmission efficiencies, how many plants in a field are already infected, and the length of the delay between initial infection and the plant becoming a CMV source for aphids.

A simulation model is being developed to forecast aphid outbreaks and spread of CMV in lupins. It calculates an index of aphid activity in the vicinity of the crop prior to the growing season. This is based primarily upon rainfall during late summer and early autumn (March and April). This determines the availability of herbaceous host plants (weeds and self-sown crop plants) on which aphids build up before moving into crops. The model then predicts the arrival of both lupin-colonising and non-lupin-colonising aphid species in the crop, their movement between plants and the spread of CMV from infected source plants within the crop. It evaluates the effects of different sowing dates, proportions of seed-infected plants and plant densities on CMV spread. Grain yield

loss and the proportion of harvested seed infected with CMV are also estimated, the latter according to cultivar. The inputs required from the model user are planting date, cultivar, numbers of established healthy and seed-infected plants per square metre, and rainfall data for the year to be examined.

The model successfully predicted the time of arrival and build up of aphids, spread of CMV, yield loss and CMV transmission into harvested seed found in four years of field experiments at Badgingarra, Western Australia. These experiments represent a range of scenarios for March-April rainfall, sowing date, level of infection in seed sown and plant density. A sensitivity analysis using the model has confirmed that the initial incidence of seed-infected plants, the established plant density and the time of arrival and abundance of aphids in lupins are major determinants of the rate of spread and final incidence of CMV in the crop, and subsequent yield loss. The model will be validated using data from different years and other sites, so as to take additional biological, climatic and cultural factors into account. The finished model is intended for use both as an educational tool and in the development of a decision support system to aid growers in the management of aphids and CMV in lupin crops.

POSTER PRESENTATIONS

MODELLING VECTOR DYNAMICS AND THE SPREAD OF INSECT-TRANSMITTED VIRUSES

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Barley yellow dwarf virus (BYDV) causes an important disease of cereals and grasses throughout the world and significant losses to crops. In the UK introduction of the disease into a field (primary infection) results from the immigration of viruliferous winged (alate) aphids into fields and subsequent spread (secondary spread) occurs as infective wingless (apterous) offspring disperse through the crop.

Pesticides tend to be applied routinely to control aphid vector populations and irrespective of the risk of virus infection. Consequently, unnecessary and ill-timed application may be made. A reliable forecasting system is needed based upon thorough understanding of the biological processes involved but until recently little was known about the factors which determine the introduction and subsequent spread of BYDV. Thus the aim of the project was to develop a computer model which predicted the spread of BYDV and which would underpin a rational Decision Support System (DSS) for both vectors and virus alike.

A stochastic individual-based simulation model has been developed integrating sub-models describing the population dynamics and behaviour of aphid vectors, and the epidemiology of the virus. The system utilises a cellular automata approach so that each individual aphid and plant within the field is monitored by the model, which utilises routines to simulate aphid development, reproduction and mortality, and the dispersion of vectors between plants and subsequent spread of virus in the crop.

The model was validated with observed data of overwintering aphid populations and virus incidence from field experiments in 1996/97, 1997/98 and 1998/99. The predicted spatial dynamics and temporal incidence of both aphids and virus were similar to those observed in cereal fields.

Furthermore the model has provided useful insights into understanding the complex interactions between biological processes involved in BYDV epidemiolgy. A sensitivity analyses of the model identified (1) virus latent period, (2) mortality of overwintering aphids, (3) the number of infective alate aphid migrants, and (4) the dispersal rate of apterous aphids within crops, as critical factors, which have a greater impact on model output than other factors. Small changes in the associated equations produced a halving or doubling of the final amount of BYDV predicted.

TEMPORAL AND SPATIAL PATTERNS OF SPREAD WITH THE NECROTIC AND NON-NECROTIC STRAINS OF BEAN YELLOW MOSAIC VIRUS IN NARROW-LEAFED LUPIN

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A new strain of bean yellow mosaic virus (BYMV), a non-necrotic strain, was found infecting narrow-leafed lupin (*Lupinus angustifolius*) in Western Australia It has become widespread in both crops and wild populations. Unlike the previous necrotic strain, which kills infected plants, it causes mottle and stunting without plant death. Plants infected with it can therefore persist, acting as sources for virus spread by vector aphids throughout the growing season. A field experiment in 1998 compared the patterns of spread of the two strains. Subterranean clover plants infected with one or other type were introduced into 20 x 20 metre plots of *L. angustifolius* (5 foci/plot, 2 plots/strain). Two plots were left without deliberately introduced foci. Within each plot, plants with characteristic symptoms of either strain were tagged on eight occasions over a 9-week period. Different tag colours distinguished tagging date and strain. At the end of the growing season, each plot was divided into 1metre^2 quadrats and all tagged plants within each quadrat counted. Grain yields were determined individually for up to 50 plants/strain for each tagging date, and for the same numbers of symptomless plants.

When BYMV spread started within the plots having 'non-necrotic foci' there were approximately equal numbers of plants infected with the non-necrotic strain to those infected with the necrotic strain in the plots with 'necrotic foci'. Subsequently, however, the non-necrotic strain spread more rapidly and by the end of the experiment there were twice as many plants infected with it as by the necrotic strain. In plots without foci, spread was also more rapid with the non-necrotic than with the necrotic strain. Overall numbers of plants infected with the non-necrotic strain tended to increase exponentially while numbers infected with the necrotic strain tended to increase linearly. With both, clustering of infected plants was greatest in the immediate vicinity of each focus. Beyond a 2.5 metre radius of each focus, there were both scattered, isolated, single infected plants and clusters of infected plants with both strains. However, clustering outside the foci was much less with the necrotic strain, which was mostly present as single, isolated infected plants or pairs of infected plants. With the non-necrotic strain, there were clusters containing up to 10 affected plants. In the plots without foci, spread of both strains was random and infection occurred mostly without clustering.

Losses in grain yield were very substantial with both strains. They were greatest with the necrotic strain, with which there was no seed production except with late infection when plants tended not to become fully infected. Yield losses with the non-necrotic strain increased with increasing duration of plant infection reaching >90% with early infection. Because of its increasingly widespread occurrence, fast rate of spread, and substantial impact on grain yield, the non-necrotic strain of BYMV is cause for concern for the lupin industry.

TEMPORAL AND SPATIAL PATTERNS OF SPREAD OF CUCUMBER MOSAIC VIRUS IN CHICKPEA

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Cucumber mosaic virus (CMV) causes a serious seed-borne disease of chickpea (*Cicer arietinum*) in Australia. A field experiment in 1997 measured the rate and pattern of spread of CMV in chickpea, a non-host plant for aphids. It also determined the losses caused when plants were infected with CMV at different growth stages. Desi chickpea cv. Tyson was sown in four 20 x 20 metre plots. Five metre wide buffers of canola (*Brassica napus*) surrounded each plot. Narrow-leafed lupin (*Lupinus angustifolius*) seed with 21% CMV seed infection was sown at five points within each of two plots to act as primary virus infection foci. The other two plots had no introduced foci. Movement by migrant aphids spread CMV. Infected plants developed CMV symptoms consisting of chlorosis, twisting and reddening of leaf tips, proliferation of axillary shoots and plant stunting. The plots were inspected four times and plants showing obvious CMV symptoms were tagged with different coloured tape on each occasion. Leaf samples from chickpea plants with characteristic symptoms were tested by ELISA to confirm CMV infection. The occurrence of infection was mapped for each of the tagging dates. At the end of the growing season, all tagged plants were harvested individually along with a nearby healthy plant for paired comparisons.

Initially most of the chickpea plants with CMV symptoms were tightly clustered around the primary infection foci. In plots with foci, spread then accelerated and within four weeks, at least half the CMV infected plants present were outside the vicinities of the foci. Spread away from the foci consisted mainly of isolated infected plants dispersed at random but formation of secondary foci had just commenced. An increase in the amount of CMV spread was evident in plots without foci at this time and this was represented by isolated infected plants dispersed at random. Two weeks later in plots with foci there was substantially greater spread away from the foci. This still consisted mainly of isolated infected plants but with greater numbers of clusters. Overall, substantially less spread occurred in plots without foci and this spread was evident as isolated infected-plants distributed at random with very few clusters. When disease progress was analysed for plots both with and without foci, there was a linear trend and not the exponential increase in spread that was expected.

Yield loss in individual plants increased with duration of infection. CMV infection caused total herbage dry weight to decrease by 53-76% and pod weight to decrease by 68-85%. Seed yield was decreased by 58-76% and the number of seeds produced was 47-69% smaller than on healthy plants. Seed size was 21-24% smaller and on CMV-infected plants there was a 10-22% increase in the amount of shrivelled grain produced.

DYNAMICAL MODELS OF HOST-PLANT INFECTION BY HELPER-DEPENDENT VIRUS COMPLEXES

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Interactions between viruses are common in nature and some viruses depend on such interactions for their survival. A virus may lack some essential molecular function that another virus provides. In so-called helper-dependent virus complexes the helper virus is transmitted independently by vectors, whereas the dependent virus is totally dependent for its transmission by vectors on molecular agents associated with the helper virus.

A general dynamical model was developed of host-plant infection by a helper-dependent virus complex. Four categories of host plants were considered: healthy, infected with helper virus alone, infected with the dependent virus alone and infected with both. The three-way interaction between virus, host plant and vector has been simplified by assuming that the vector infectivity mirrors host infection. New plantings of the host crop were constrained by a maximum abundance imposed by an assumed limitation of cropping area. A number of alternative equilibrium states of host infection could occur determined exclusively by the values of parameters and it was informative to display their distribution in the parameter plane where A, the ratio of infection rate / host loss rate due to infection, is proposed as an important epidemiological quantity, the *mutual* adaptation of the virus and the host plant.

Analyses of the distribution of the final equilibria illustrated the following. (i) A well adapted helper virus increased the opportunity for a dependent virus to evolve and survive. The model therefore explained why infection with a helper virus usually causes no or little damage to plants, whereas infection with a dependent virus or mixed infection with both often causes very severe damage. (ii) It is clear that the helper virus affects the survival of the dependent virus, but it was also found that the dependent virus could affect the survival of the helper and so the synergistic association could be reciprocal. (iii) The model also predicted a situation in which it was possible for a very badly adapted helper virus to survive provided the dependent virus was very well adapted. Though theoretically possible, this state is not know to occur.

SMPVY: A SIMULATION MODEL FOR POTATO VIRUS Y INFECTING PEPPER (*CAPSICUM ANNUUM* L) CROPS

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The SMPVY is a simulation program that predicts the progress and incidence of the infection caused by potato virus Y in open-field pepper crops. The model uses data obtained from aphid trap

catches in green tile traps located in different pepper growing areas during four years: 1991-1994. The program considers the vector efficiency of new aphids alighting in the field for the first time (primary infection). The aphid species considered are the most abundant and/or efficient transmitting PVY to pepper crops grown in Spain. These species are *Aphis fabae*, *Aphis* spp., *Brachycaudus amigdalinus*, *Diuraphis noxia* and *Myzus persicae*. The model describes the infection of PVY dynamically and considering its spatial distribution, and the primary and secondary spread. Finally, the program determines the yield loss due to PVY as a function of the time of virus inoculation.

The program allows setting two different types of users: a standard user and an advanced user mode. The standard user mode allows entering a limited number of data and initial conditions. The advanced user mode allows entering and modifying default values of standard parameters that have been set by the program. The default values used are mean values obtained from research conducted under certain conditions and may vary locally and annually depending on the climate of the region under study. Therefore, the advanced user can modify and set these default values to the particular conditions and seasonal climatology of the region under study. SMPVY can provide information and predictions on yield loss expectations due to PVY infection and therefore, may be used as a decision-making tool for disease management.

SMPVY runs under Microsoft Windows and has a user-friendly interface with graphic and numeric presentation of the simulation outputs. SMPVY is currently being validated using data from PVY epidemics occurring in the Central Region of Spain during the period 1995-1998. The model will be adjusted depending on the results obtained from the validation process.

SPREAD OF POTATO VORUS Y IN POTATO FROM AN EXTERNAL SOURCE: SPATIAL AND TEMPORAL PATTERNS

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The control of viruses in seed potato production relies mainly on preventive measures, one of them being isolation from external sources of infection. In 1995, Basque seed growers were allowed to produce their seed under a ìmixed areaî scheme, i.e. interspersed with ware fields. This provided the conditions for studying potato virus Y (PVY) infection from adjacent fields.

Four farmersí seed potato fields planted in the vicinity of ware fields, plus a control consisting of a seed field isolated by eighty meters of cereal, were tested at different times in the growing season for PVY infection. Samples were taken at different distances from the field edge. After maturity, samples of tubers were also analyzed. Three of the four fields planted in the vicinity of ware crops showed an exponentially decreasing spatial gradient of infection from the edge inwards. The fourth one and the control field showed no such gradient.

The disease progress curves were of sigmoid shape with upper asymptotes lower than 100% infection in all the sampled rows. The availability of healthy plants was not, therefore, the a factor limiting disease progress. The disease increased concurrently, although at different rates, at all distances from the field edge. All this could be interpreted as the infection having been produced concurrently in

all rows by the main aphid flight peak (end of June) and showing up in the plants two weeks later. As the aphids moved further away from the external infection source, they had lost their infectivity by probing on more and more uninfected plants.

In all the cases the harvested material far exceeded the tolerances for seed certification. The ìmixed areaî scheme is thus to be discouraged.

TOTAL APHID FLIGHT MONITORING BY YELLOW WATER TRAPS: STEPS TOWARDS MODELLING.

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Non-persistent viruses, including potato virus Y (PVY: *Potyvirus*) have limited vector specificity, being transmitted by many species within the *Aphidinae*. Monitoring the flight of all the aphid species collectively (*Homoptera: Aphididae*) could be a "quick and dirty" approach to risk assesment, since no individual identification would be necessary.

A data set of aphids caught in yellow water traps for 20 years in the Basque seed potato growing area was studied by analysis of variance techniques. The first approach was a factorial design of Year x Week x Location to analyze the log transformation of the total aphid captures, using the triple interaction term as the experimental error. The main factors accounted for most of the variability, but the Year x Week and Year x Location interactions were also highly significant. This means that eventual forecasts should be built in a year-to-year basis.

This explanatory model was further refined to a third-degree polynomial over the weeks plus a fixed effect for Location, both nested within Year. It gave a good fit, except for 1993 and 1996, when the flight dynamics curves were flat. The polynomial models flight dynamics with a peak in June and a trough in August. The peak date changed from year to year, but the delay from the peak to the trough was fairly constant, *c.* 11 weeks. Any relationships of the flight peak height or date with meteorological variables were not obvious.

MODELLING VIRUS EPIDEMIC DEVELOPMENT USING LONG-TERM STATISTICS

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Plant virus pathosystems, and interactions with vectors and hosts are often very complex and are affected by different factors. These are biotic (presence and prevalence of vectors, weeds, anthropogenic pressure) and abiotic (air and soil temperature, humidity, day length, wind velocity etc.). Development of multifactor models requires a knowledge of the influence of all above-mentioned factors on viral population development. Obviously, these models are good only in artificial systems with a restricted number of monitoring parameters. A more complex approach to modeling viral population dynamics is proposed. We have observed fixed number of agrocenoses in different regions of Ukraine for several years. These regions were selected so that they differed in climate characteristics, anthropogenic pressure, and ecological complexity. Monitoring of 16 of the most harmful agricultural viruses was done using ELISA, electron microscopy and visual tests. A database which incorporated original processing methods was created. Received data after subsequent processing provides dynamic estimations of virus spread on different ecosystem levels. At the first stage by comparison of virus antigen presence simultaneously in soil and crops, in soil and weeds, weeds and crops we can define viral transmission ways, its hosts and reservoirs. Comparing simultaneous presence of two or more viral antigens can demonstrate dual infection. At the second stage we can define virus population dynamic parameters as affected by crop rotation. Finally we can propose complex solutions to prevent epidemic development by optimal crop rotation. Such methods can greatly reduce the costs of field maintenance by decreasing usage of herbicides and pesticides.

TEMPORAL AND SPATIAL SPREAD OF TOMATO SPOTTED WILT TOSPOVIRUS IN RELATION TO THRIPS POPULATIONS IN TOBACCO CROPS IN NORTHERN GREECE

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Temporal and spatial spread of tomato spotted wilt tospovirus (TSWV) were studied in tobacco fields of cv Virginia in Kilkis, one of the most important tobacco producing areas of Northern Greece. The study was performed in two experimental plots during 1995, one in 1996 and one in 1997. Sampling of thrips and infected plants was performed biweekly, both in the seedbeds and in the field. Thrips population was recorded using blue sticky traps (Horiver-TR). In seedbeds, TSWV incidence was estimated by testing 100 randomly selected leaves by ELISA using antibodies against the N protein of TSWV (BR-01). In the field, plants showing typical symptoms of TSWV infection were counted, their position in the plot was marked and ten of them were randomly collected and tested by ELISA to confirm infection. The peak of thrips population was recorded

during the 22 nd and 23 nd week of the year 1995 in both plots, reaching approximately 5000 adults per trap. At the end of the period only 7.4% and 2.2% of plants were infected in the two plots. During 1996 the peak of thrips population was in the 18th and 19th week, whereas in 1997 it was recorded two weeks later (20th and 21st week), reaching 1400 and 1800 thrips per trap, respectively. At the end of the season, in both years, all plants were infected by TSWV. The coordinates of the infected plants placed within the rectangular area were determined based on a defined coordinate system. Then it was tested whether these points were scattered randomly by using a distanced-based Monte Carlo test. The statistic used for the Monte Carlo test was the mean of the distance between plants and their nearest neighbors. The test was performed for the data of each sampling day**.** The statistical analysis revealed that the pattern of infected plants in not random ($p<0.01$).

Thrips identification showed that *Thrips tabaci* constituted the predominant thrips species and the only vector of TSWV collected on the traps. Other species such as *Aeolothrips* spp., *Limothrips cerealium, L. Denticornis, Taeniothrips* spp. *Thrips* spp., which are not known to be vectors of TSWV, were also identified.

SPREAD OF BEET MOSAIC POTYVIRUS AFTER INOCULATING THE CROP AT DIFFERENT DATES.

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Beet mosaic virus (BtMV) is, as all other viruses of the genus *Potyvirus*, transmitted non-persistently by different aphid species with variable efficiencies. Outbreaks of BtMV in Netherlands could not be correlated with the population dynamics of any specific aphid species. Alatae migrants are believed to play a major role in the spread of potyviruses due to their short acquisition periods, active probing behavior and active search for a new host. The relationship between aphid population and spread of potyviruses has been studied by different approaches. The only general conclusion drawn from these studies is that the development of the alatae aphid population overlaps the disease progress curves, and that a single species is not directly correlated to the spread of potyvirus in a single pathosystem.

Field experiments were conducted in 1995 and 1996 to study the development of BtMV epidemics after inoculating field plots at different dates. The development of the infections was recorded weekly or fortnightly after inoculation. The relationship between the epidemics and alatae aphid population was studied by means of multiple regression analysis, Pearson product correlation and a mechanistic simulation model. Total daily aphid population caught by a suction trap proved to be the best parameter to explain the developed epidemics studied. Introduction of the disease before the aphid flight resulted in similar epidemics. In the simulation studies, spread of BtMV could be related to the aphid population by a single intrinsic rate. Simulated epidemics at different inoculation dates fit the experimental data, supporting the use of this single rate to link aphid population and spread. Further improvement in the model may focus on the species composition of the aphid catches and the aggregated spatial pattern of the patches with infected plants.

SESSION 5

EPIDEMIOLOGY OF ARTHROPOD-BORNE VIRUSES

ORAL PRESENTATIONS

TOMATO SPOTTED WILT TOSPOVIRUS AND VECTORS IN PORTUGAL

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Tomato spotted wilt virus (TSWV) is the type-member of the genus *Tospovirus* currently includes 12 other members which are important pathogens of vegetables and ornamentals in greenhouse and field crops. Tospoviruses are transmitted in a persistent-propagative mode by thrips (Thysanoptera, *Thripidae*), the known field vectors. *Frankliniella occidentalis*, the most efficient vector worldwide, was detected in 1989 for the first time in Portuguese greenhouses on imported rose and gerbera (Entre-Douro e Minho region) (Guimarães & Lopes, 1990) and thrips are currently important pests in many crops. TSWV was detected for the first time in Portugal (Alentejo-Odemira region) in 1990 from imported ornamentals (Louro, 1991) and since 1991 the thrips/TSWV complex appears to be established in several regions.

In 1997, we started a national project to study the epidemiological complex tospovirus-vegetables and ornamentals in greenhouse and fields crops. Monitoring and identification of thrips from the north and south of Portugal in several greenhouses and field crops is in progress. *Frankliniella occidentalis*, *Thrips tabaci* and *Frankliniella intonsa* have been identified of the known tospovirus vectors. TSWV acquisition and transmission capacity by *Frankliniella occidentalis* biotypes is being assessed using biological assays monitored by ELISA (squash-blot and dot-blot, respectively).

A serological study (DAS-ELISA or Dot-ELISA) was done on symptomatic field plants, with polyclonal anti N-sera to the tospoviruses transmitted by the possible vectors identified. Samples from vegetables and ornamentals reacted positively only with the TSWV antiserum. Several weeds near infected field crops were also reservoirs for the virus and the thrips. Biological and molecular characterization of Portuguese isolates from pepper and tomato are in progress. Molecular studies confirmed the high homology of our isolates to TSWV. The host range of these Portuguese isolates slightly differs from the known reaction produced by TSWV on the same hosts, namely by inducing systemic infection in hosts that are referred as just developing local symptoms for TSWV.

INCIDENCE AND EPIDEMIOLOGY OF CITRUS TRISTEZA VIRUS IN THE VALENCIAN COMMUNITY OF SPAIN.

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The Valencian Community (VC) is one of the main citrus areas (184000 ha, 83 million trees) in the world. The first outbreak of tristeza disease caused by citrus tristeza virus (CTV) was recorded in 1957 and since then more than 35 million trees (mainly sweet oranges and mandarins) grafted on sour orange have declined. From 1957 to 1989 *Toxoptera auranti* and *Aphis spiraecola* (poor vectors of CTV) have predominated (14% of CTV incidence in 1989). In the last 10 years the more efficient vector, *Aphis gossypii*, has become predominant inducing a fast spread of the disease (46% average infected trees in 1997). The incidence of CTV and its progress is known in the most important municipalities in growing citrus in the VC. Approximately 2% of the trees have been analysed in large-scale surveys in the last 10 years. Models of spatial and temporal spread of the disease have been established in different areas (Gottwald *et al*; 1995. Phytopathology. 86, 45-55). The differences between the temporal spread of CTV in clementina de Nules mandarin (logistic curve) and sweet orange Washington navel (Gompertz growth curve), have been evaluated in the same area. The susceptibility of the different species or varieties to natural infection seems to depend on the number of shoots and on the period of time in which they remain succulent. Accordingly, the most easily infected rootstocks are Cleopatra mandarin and *Citrus macrophylla*.

Correct inform ation on the CTV isolates presents their incidence and epidemiological behaviour has been essential for a better management of tristeza disease in Spain. These data and knowledge have allowed specific recommendations to growers in order to reduce CTV damages and to make decisions concerning the time for the trees to be grafted on sour removal. New plantations with certified plant material free of viruses and grafted on CTV-tolerant rootstocks represent 80% of the plantations in the VC. The CTV problem would be solved if more aggressive isolates were not introduced.

SPREAD OF RICE YELLOW MOTTLE SOBEMOVIRUS AS DEDUCED FROM FIELD OBSERVATIONS AND EXPERIMENTAL RESULTS

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Rice yellow mottle virus (RYMV) causes severe infections mainly in irrigated rice, whereas infections are generally absent in dry-land rice. The virus was first reported in Kenya, but is now known to occur in almost all irrigated rice growing areas in Africa. The virus is transmitted by beetles and mechanically. Infected plants are rarely found in the seedbed, but usually occur two to three weeks after

transplanting the seedlings. Different patterns of spread can be observed in and between the infected fields. Patches vary in size from a few infected plants to areas with a diameter of 10 m or more. Completely infected fields are also encountered. A gradient of decreasing symptom severity can often be discerned around severely infected plants. The observed patterns of spread cannot be attributed to transmission by beetles only. Spread by this transmission would result in a more even and scattered distribution of singly infected plants or of small patches of infected plants. The observations made, experiments done and the number of beetles caught show that most of the spread is not due to transmission by beetles but is caused by mechanical inoculation. Most infections are the result of exposing healthy seedlings and plants to virus-contaminated and infected material (water, soil, cattle faeces and plants).

The first infections may occur in the seedbed. These seedlings will produce infectious guttation water that contaminates the seedbed water. These infected seedlings may also infect neighbouring plants when disturbed by wind. During transplanting, the farmer will inoculate the seedlings with his hands contaminated by the guttation water, seedbed water and infected seedlings. Further spread of the virus will occur by touching the plants while weeding and other activities in the crop. Plants can also become infected from virus released into the soil.

Harvesting the crop may lead to further spread of the virus by the implements used. After the harvest, the virus can be spread further by grazing livestock. Infectivity of the virus has been demonstrated in the faeces of cows and, hence, manure may become a source of infection. Fields without any infection history became infected when herds were allowed to overnight. In case an infection is established in a field, virus will be released from the roots and stubbles when the field is prepared for the next crop. The observation was made that surface water appeared still to be infectious three weeks after ploughing a recently planted crop which appeared to be completely infected.

EPIDEMIOLOGY OF THE CARROT MOTLEY DWARF VIRUS COMPLEX IN PARSLEY

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For more than 15 years carrot motley dwarf (CMD), (a virus disease transmitted in a persistent way by the willow-carrot aphid (*Cavariella aegopodii* Scop.), has been an important parsley disease in Belgium. The CMD virus complex consists of three components: carrot red leaf luteovirus (CRLV), carrot mottle umbravirus (CMoV) and CRLV-associated RNA (CRLVaRNA). So far, there is no rapid and sensitive detection method for the three components in parsley, little or nothing is known about the epidemiology of CMD in parsley and it is still difficult to control the disease. In this epidemiological study the aphid vector symptom expression on parsley (yellow and red discoloration of the leaves, stunting) were investigated during 1997 and 1998. The flights of the aphid vector to parsley were monitored and revealed a clear flight pattern for *C. aegopodii* with three different flight peaks. Although the timing of the flights in 1997 and 1998 was similar, the intensity of the flight peaks was totally different. To study the population of *C. aegopodii* on parsley and other secondary hosts (umbelliferous weeds), a sampling procedure and an extraction method for aphids was developed and evaluated. Using this methodology, the population of the aphid vector on parsley was studied and revealed important differences between 1997 and 1998. The most important

umbelliferous weeds colonised by the aphid vector were determined. The presence of CMD symptoms in two different plantings of parsley was investigated and compared using a visual scale. There was no correlation between the presence of the aphid vector on parsley and the incidence of CMD symptoms. Surprisingly, 1997 (low number of early flying *C. aegopodii*) gave a significantly higher percentage of parsley plants with specific symptoms than 1998 (very large number of early flying *C. aegopodii*). The influence of environmental factors on CMD symptom development in parsley is discussed.

EPIDEMIOLOGY AND CONTROL OF WHEAT DWARF

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Wheat dwarf is a sporadic, yet serious, disease of winter wheat. The causal agent is wheat dwarf virus (WDV), a geminivirus transmitted by the leafhopper *Psammotettix alienus*. The disease has during the last decade been reported from several countries in Europe. In Sweden, severe outbreaks occurred early in the century and in the 1940s. In 1997, WDW was again common and caused yield losses of up to *c*. 80 %.

In this study we quantified the rate of virus spread in autumn, by leafhopper adults immigrating into winter wheat fields, and in the following summer, by their progeny. We also examined the possibility of forecasting damage and preventing yield losses by cultivation measures or chemical control of the vector. During 1998 nine winter wheat fields were surveyed for virus incidence and vector occurrence. Wheat plants were sampled and tested serologically by ELISA in May, before the hatching of overwintered leafhopper eggs, and then, in most cases, every second week until the middle of July. Leafhoppers were monitored by using yellow water traps. Farmers who applied a pyrethroid spray in spring left an untreated area of *c*. 0.5-1 ha.

Infected plants were found in most of the surveyed fields in spring, but the incidence was low (at most 5 %). The first leafhopper nymphs started to occur in mid-May and adults appeared in early June. In some of the fields virus incidence increased sharply in mid-June, indicating that a significant spread of virus occurred during early summer. The final virus incidence in each field was more related to the initial rate of infection than with leafhopper catches. Chemical control in spring had a limited effect; at the most it halved the proportion of infected plants compared with unsprayed areas.

The large secondary spread of virus implies that it should be possible to avoid damage by spraying in spring if control effectiveness can be improved. Compared with autumn control, this increases the possibilities to forecast damage based on vector and virus surveys.

CHARACTERIZATION OF IRIS YELLOW SPOT TOSPOVIRUS AND ITS TRANSMISSION BY THRIPS

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In December 1996 unusual viral symptoms of chlorotic spots and rings on *Hippeastrum* leaves were observed in the Jordan Valley and the Besor area. The virus produced symptoms on *N. benthamiana, C. quinoa* and *G. globosa* but not in other test plants. Electron microscopy (EM) using ultrathin sections of infected tissues of *Hippeastrum* and *N. benthamiana* revealed tospovirus-like particles. The virus was identified as IYSV using an antiserum kindly provided by D. Peters (Wageningen, the Netherlands).

During October 1997, 20-60% of field-grown onions (*A. cepa*), with unusual viral symptoms of straw-colored ringspots on leaves and flower stalks were observed in Bet Shean Valley, Israel. Leaf samples were analyzed by transmission EM of leaf dip preparations. Typical tospovirus-like particles were observed only with samples taken from symptomatic plants. Crude sap from symptomatic tissue was transmitted mechanically to *N. benthamiana*, *C. quinoa,* and *G. globosa*. On inoculated plants of *N. benthamiana*, chlorotic spots developed on inoculated leaves followed by systemic necrosis 5 and 10 days post-inoculation (DPI), respectively. On inoculated plants of C. *quinoa* and *G. globosa*, necrotic local lesions developed by 4-5 DPI. EM studies using ultrathin sections of infected onion and *N. benthamiana* leaves revealed tospovirus-like particles. Virus was purified from mechanically infected *N. benthamiana* and identified as IYSV by western blots and ELISA (anti-IYSV antiserum was provided by D. Peters, Wageningen, the Netherlands). The high incidence of the disease observed in the surrounding fields and in other onion growing areas in Israel was associated with large populations of thrips. In surveys of thrips populations and IYSV incidence in onion, *Thrips tabaci* predominated and its population abundance was significantly correlated with IYSV incidence. In order to verify the role of onion thrips in virus spread, field-collected individuals were placed on onion disks. The high rates of transmission that were obtained reflect the high proportion of viruliferous insects in the population. The utility of field assessment of thrips activity in IYSV transmission is discussed.

EPIDEMIOLOGY OF POTYVIRUSES INFECTING MAIZE IN MEDITERRANEAN BASIN, SPAIN

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Commercial maize fields in northeast Spain were surveyed for maize dwarf mosaic and sugarcane mosaic potyviruses during two consecutive years. Samples were analysed by DAS-ELISA using polyclonal antisera against these viruses.

Maize dwarf mosaic virus (MDMV) occurred in all surveyed fields; its average incidence in randomly sampled maize was 21.6% and 99.6% in plants having samples. In contrast, sugarcane mosaic virus (SCMV) was found only in one field and its incidence was <0.6%. These results are consistent with the view that MDMV is the most prevalent potyvirus infecting maize in Mediterranean countries.

Sorghum halepense seems to be the only host that plays a significant role in the epidemiology of MDMV. The abundance of this grass, which was found in almost all the fields, together with the high percentage (57%) of samples infected by the virus can explain the high incidence of MDMV in maize. Besides, it has been found that this grass is a source of variability for MDMV and that the virus survives in it from season to season.

No evident alternative host was found for SCMV. However the other four most abundant weeds found in the survey (*Bromus*, *Brachypodium*, *Cynodon* and *Setaria*) have been reported as experimental hosts of SCMV.

EPIDEMIOLOGY AND MANAGEMENT OF TOMATO SPOTTED WILT TOSPOVIRUS

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Diseases caused by tomato spotted wilt *Tospovirus* (TSWV) are a major constraint to the production of peanut (groundnut), tomato, pepper and tobacco in Georgia. The virus is transmitted by western flower thrips (WFT), *Frankliniella occidentalis* and tobacco thrips (TT), *F. fusca*, which are prevalent in Georgia. Prior research has shown that peanut supports the reproduction of these two vector species, whereas little vector reproduction was observed on tobacco. The virus must be acquired at the larval stage for the adult to transmit the virus. Detection of NSs (a non-structural TSWV protein present only following virus replication) in thrips by ELISA is a reliable indicator that the virus has multiplied in the vector and thus the vector is competent to transmit TSWV. The proportion of thrips that were potential transmitters in the field populations of WFT and TT collected from peanut fields was determined by assaying for Nss. It was shown previously that TSWV replicates in WFT. Immunological evidence was obtained for the first time demonstrating that TSWV also replicates in tobacco thrips. The prevalence of potential transmitters in TT and WFT varied during the peanut growing season. After an initial peak in April, the highest number of potential transmitters was detected during May and June. Both TT and WFT transmitters were detected in April whereas TT was the predominant vector during the later part of the season. Thrips positive for NSs were not found in either species after late September. While TT was the predominant vector during 1996, more WFT were found during 1997. Overall, 8.1% of TT and 3.4% of WFT were found to be potential transmitters as assayed by the NSs-specific ELISA. Additionally, larvae collected from volunteer peanut plants were reared to adults in the laboratory without any access to TSWV and the resulting adult TT and WFT were assayed individually by ELISA. NSs was detected in adults of both TT and WFT indicating the potential role of volunteer peanut plants in disease spread.

Molecular characterization of various TSWV isolates infecting peanut, tobacco, tomato, pepper, watermelon and stokesia showed that the nucleocapsid gene sequences were highly conserved. Phylogenetic trees showed close clustering of Georgia isolates indicating the effect of geographic distinctiveness on sequence evolution of TSWV populations.

Recent field studies showed that the use of certain insecticides and growth promoters alone or in combination had significant reduction of final disease incidence in tobacco, tomato and peanut. Data obtained suggest that an integrated disease management strategy utilizing cultural and chemical methods combined with the use of virus-resistant cultivars has a potential to minimize the impact of spotted wilt in field crops.

POSTER PRESENTATIONS

EPIDEMOLOGICAL STUDIES OF VIRUS DISEASES OF FABA BEAN IN EGYPT

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Leguminous crops are an important protein source for human diets in many parts of the world. Within Egypt, *c*. 330,000 acres of faba bean *(Vicia faba* L.) are cultivated and the major limitation to successful production of this crop is virus infection. The objective of this work was designed to study the epidemology of the most economically important viruses of faba bean in Egypt, followed by isolation and identification of these viruses.

Samples of faba bean plants with symptoms suggestive of virus infection were collected from 163 fields in Upper, Middle and North Delta in Egypt. These samples were transferred directly to the lab for detection by DAS-ELISA technique using antisera to alfalfa mosaic virus (AMV), broad bean mosaic potyvirus (BBMV), broad bean stain comovirus (BBSV), broad bean wilt fabavirus (BBWV), pea leaf roll luteovirus (PeLRV), bean yellow mosaic potyvirus (BYMV), cucumber mosaic cucumovirus (CMV) and faba bean necrotic yellows virus (FBNYV). Mechanical and aphid transmissions were attempted. Antisera raised against both FBNYV and BYMV were prepared and ELISA Kits were produced.

Seven viruses were detected:- FBNYV, BYMV, BBSV, BBMV, BLRV, BBWV and CMV - in samples collected from the open fields in different regions. FBNYV was found to be the most common virus with an incidence of 37, 43 and 33% followed by BYMV (22, 24 and 20%) and BBSV (16, 9 and 14%) in the tested samples of Upper, Middle and North Delta, respectively. According to these results, FBNYV, BYMV and BBSV were the most widespread. Therefore, these three viruses were isolated and subjected to further studies. FBNYV was not transmitted mechanically, but was succesfully transmitted by aphids. *Acyrthosiphon pisum* (Harris) was the most effective aphid (93%) followed by *Aphis craccivora* (Koch) (60%) and *A. fabae* (Scop) (33%). BYMV was mechanically and aphid-transmitted (non-persistent manner). and the percentages of aphid transmission were 45-90%, while BBSV was mechanically and seed transmitted with percentage of <2%. Isometric (18 nm in diameter) and filamentous (770 x 13 nm) virus particles were purified from faba bean leaves infected with FBNYV and BYMV, respectively, and polyclonal antibodies were raised against them and ELISA KITS were produced and used successfully for virus detection for the first time in Egypt.

BROAD BEAN WILT FABAVIRUS INFECTING MEDICINAL AND AROMATIC PLANTS IN ITALY

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Medicinal and aromatic plants are becoming more and more economically important in Italy. A survey was made between 1990 and 1998 at the Herb Garden of Casola-Valsenio and of some experimental crops located in Emilia-Romagna region (northern Italy), to identify the most frequently occurring viral infections and to determine their incidence for production. Several plants were found showing virus-like symptoms and they were tested by mechanical inoculations on herbaceous indicator plants of the families: *Amaranthaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Labiatae*, *Leguminosae*, *Solanaceae*; electron microscopy; protein A sandwich enzyme-linked immunosorbent assay: PAS-ELISA). Eight species were infected by broad bean wilt fabavirus (BBWV), alone or in mixed infections with other viruses. The sera used in PAS-ELISA tests were those to BBWV serotypes I and II (provided by the Istituto di Fitovirologia Applicata, CNR, Turin, Italy).

BBWV-I infected: *Borago officinalis* (stunting of the plants; systemic mosaic, yellowing, crinkling and malformations of the leaves; dwarfing and colour breaking of the flowers); *Digitalis lanata* (chlorotic mosaic and necrotic stripes on the leaves; turnip mosaic potyvirus: TuMV, was also present in some plants); *D. purpurea* (leaf crinkling); *Hedysarum coronarium* (leaf yellowing); *Leonorus cardiaca* (chlorosis and yellow spots on the foliage); *Phytolacca decandra* (yellow and necrotic spots on malformed or crinkled leaves); *Polygonum fagopyrum* (chlorotic mosaic on the leaves; mixed with TuMV); *Valeriana officinalis* (yellow mosaic on the leaves and stunting of the plants; in some of these, also showing leaf and stem necrosis, cucumber mosaic cucumovirus: CMV, was present). BBWV-II occurred in *D. lanata* showing a systemic chlorotic mosaic.

This investigation demonstrates that BBWV naturally infects several medicinal and aromatic plants, causing severe symptoms which can reduce the yield of the crops. This virus is more prevalent in Italy than is generally believed. BBWV-II, not found previously in Italy on medicinal and aromatic species, is also present. The damage caused by this virus appears serious enough to require control measures, such as: removal of infected plants and elimination of weeds and aphids (natural vectors not only of BBWV, but also of TuMV and CMV). In particular, BBWV-I was found in *Plantago lanceolata* and in other unknown weeds at the Herb Garden of Casola-Valsenio. To prevent virus infections the crops should be located in exposed mountain areas of high rainfall, where aphid infestations are unlikely.

VIRUS DISEASES OF LAVENDER PLANTATIONS IN CRIMEA

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On industrial lavender plantations in the Crimea, diseased plants occurred having previously unknown symptoms. The leaves became yellow and rolled, chlorosis appeared on the plant (shoot apices discoloured) and after 2-3 years the plants dried up. On other plants degeneration of shoots and their untimely drying up were observed. The diseases became apparent at the end of summer and in autumn.

On different lavender plantations damage affected 18-80% of all plants. Flowering of diseased plants decreased in the second year. In some years the spread and damage caused by lavender virus disease with such symptoms was observed in the regions of growth in Crimea. Isolates were obtained from diseased plants and inoculated to indicator plants. Indicator plants inoculated with sap of diseased plants having symptoms of chlorosis and leaf roll developed characteristic lesions in the form of light and dark areas of necrosis. *Gomphrena globosa* L., *Chenopodium amaranticolor* L. and *Chenopodium quinoa* L. accumulated viruses best of all.

The disease peaks at flowering time. During the investigations the infectious nature of the disease was proved. Electron-microscopic analysis of the sap of diseased plants showed viral particles resembling potyviruses and luteoviruses.

Testing of plants which were taken from different regions of Crimea gave different results. In the mountainous areas there was a low incidence of damaged plants, but in the places of intensive agriculture the incidence reached 60-83%.

RICE YELLOW MOTTLE VIRUS DISEASE IN TWO RICE CROPPING SYSTEMS OF TANZANIA

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The epidemiology of rice yellow mottle virus (RYMV) is currently the least understood problem of rice production in Africa. In Tanzania the virus has been identified in all major rice growing regions, yet the causes of severe RYMVD outbreaks are unknown. During the course of an epidemiological study of RYMVD in Tanzania, surveys were carried out to determine if there are differences relating to the incidence of RYMV in the major rice production systems in Tanzania - rainfed lowland bunded (Mwanza) and rainfed lowland shallow flooded (Morogoro).

In Mwanza, weeds are ploughed into the soil during land preparation and rice seedlings are usually transplantated, whereas in Morogoro, land is cleared (slash and burn) before ploughing and seeds are broadcast onto the prepared land. The most popular variety grown in both systems is known as Supa, a traditional, low-yielding variety which produces aromatic grain. In Morogoro, however, improved rice varieties such as Line 88 and 85, have been introduced and adopted by local farmers.

In 1997 RYMVD was more prevalent in the unbunded, shallow flooded areas of Morogoro and large areas of the crop were affected by RYMVD. The presence of RYMV in these fields was confirmed by serological tests, but no beetle vectors were found. This pattern of disease was not observed in the bunded areas around Lake Victoria, near Mwanza where, in 1997 and 1998, RYMVD was only observed in small patches within or at the edge of fields. In this region, however, low numbers (<1 adult per 10 fields) of a known vector (*Trichispa sericea*) were collected from fields containing plants 20-45 days old.

Gel-diffusion tests revealed serological differences between the RYMV strains found in Morogoro and Mwanza. Studies are being carried to find out if these two strains differ in their symptom expression on three rice varieties. Three lines of rice were used - Supa, Line 85 and one developed by WARDA which is presumed to be resistant to the West African RYMV strains. Preliminary results indicate that Line 85 and Supa are susceptible to both Tanzania strains, whereas no virus was detected in the line developed by WARDA.

PREVALENCE OF VIRUS DISEASES AND THE INCIDENCE OF YAM MOSAIC VIRUS IN YAM (*DIOSCOREA* sp.) FIELDS FROM BASSAR AND SOTOUBOUA PREFECTURES OF TOGO

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Yam (*Dioscorea* sp.) is an important food crop which contributes to the diet of several million people in the tropics. However, persistent constraints such as virus diseases could reduce considerably the yield of this plant, causing 27 to 41% or more loss in some cases. Yam mosaic virus (YMV) is particularly widespread in some Togolese yam-growing areas but the prevalence of virus diseases and the incidence of YMV are still unknown in Bassar and Soyouboua, two major yam-producing prefectures. The present study was undertaken to gain more information on the epidemiological aspects of this virus. Surveys were conducted through 25 and 18 yam fields, respectively, in Bassar and Sotouboua and the severity of yam virus diseases was assessed as well as YMV incidence. Immuno-enzymatic method TAS-ELISA (with monoclonal and polyclonal antibodies) was used to identify YMV in 293 foliar samples from the two prefectures. The results obtained indicated that the incidence of yam virus diseases in both prefectures were 100% and that the virus symptoms (mosaic, mottling) were very severe in Bassar and Sotouboua, respectively, on 16 and 17% of infected yam plants. The incidence of YMV was 44% in Bassar and 32% in Sotouboua.

SOME OBSERVATIONS ON MAIZE STREAK DISEASE AS A CONSTRAINT OF MAIZE PRODUCTION IN WEST KENYA

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Maize is the major food crop in Kenya. The average annual maize production estimate for 1991 was 3.3 million metric tonnes in an area of 1.5 million hectares. The gap between intended and actual yields ranges from 2 tonnes/ha in lowlands to 5 tonnes/ha in the highlands. This low production per unit area has been attributed to various factors including low rate of adoption of technical recommendations, diseases and pests. A limited survey of maize streak virus disease in west Kenya during the long rain season of 1998 showed that disease incidence ranged from zero to 50 percent. High incidences of disease were observed in Homabay (27%), Kuria (35%) and Teso (52%) districts. In most places maize streak disease was associated with hybrid varieties (H 614D, H 625, H 512 and Pwani hybrid 1). Invariably, the varieties H 614D and H 625 recommended for highlands were also popular in the mid-lowlands where they exhibited high disease incidence. In disease-affected areas some farmers attributed the disease to either tobacco smoke or nutrient deficiency. The possible reasons for the high maize streak disease incidence and the implications for disease management in Kenya are discussed.

PRESENT STATUS OF VIRUSES OF VEGETABLE CROPS IN SOUTHERN ITALY

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In the last three years the following viruses, listed in decreasing order of current economic importance, affected vegetable crops in southern Italy: tomato spotted wilt (TSWV), cucumber mosaic (CMV), zucchini yellow mosaic (ZYMV), tomato yellow leaf curl (TYLCV) and impatiens necrotic spot (INSV). In addition to the above-mentioned viruses, increasing incidences of potato virus Y (PVY), alfalfa mosaic virus (AMV) and pelargonium zonate spot virus (PZSV) were reported in canning tomato crops.

Since its first appearance in 1990, TSWV infects recurrently all main vegetable crops (tomato, pepper, eggplant, lettuce, chicory) in southern regions and recently it has been recorded in artichoke, a perennial crop thus leading to unpredictable consequences on the epidemiology of the virus. In 1997, incidence of TSWV in tomato increased unexpectedly from 15 to 95% with devastating consequences on both tomato production and canning industry. In the same year INSV, a virus previously confined to ornamental crops, was detected in lettuce and chicory. These observations seem to support the hypothesis that dramatic changes are occurring in viral populations of both TSWV and INSV. The outcome of an extensive survey initiated in 1998 could help to elucidate the changing aspects of virus/vector/host relationships in southern Italy.

Since 1988, the economic importance of CMV in southern Italy correlates with recurrent outbreaks in canning tomato crops. Although its current status ranks second after TSWV, the virus remains a threatening pathogen, hampering cultivation of tomato in all areas where epipidemics

occurred. Continuous survey of highly infected areas do not show any relevant change in resident virus population.

TYLCV was first reported in 1988 in Sardinia, later in Sicily and in 1991 in Calabria (southern Italy). Although an increasing number of reports was anticipated some years ago since the whitefly vector, *Bemisia tabaci*, occurred in many Italian regions, current records suggest the virus is still confined to insular Italy and Calabria. However, recent observations report, unlike in the past, populations of vector stably adapted to tomato crops. Tomato and probably *Solanum nigrum* are likely to be the main source of TYLCV for successive cycles.

Details of the present status of ZYMV, PVY, AMV and PZSV infections in vegetable crops and current virus management strategies will be also reported.

This work was supported by a grant of the University of Bari "Studio epidemiologico di tospovirus su colture ortensi e ornamentali e caratterizzazione biologico-molecolare di ceppi necrotici del virus del mosaico dell'erba medica (AMV).

ORNAMENTALS, WEEDS AND THRIPS FAUNA ASSOCIATED WITH TOSPOVIRUSES IN GREECE AND SOUTHERN ITALY

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A survey was conducted in order to record the ornamental plant-hosts of tospoviruses in Greece and Southern Italy (Apulia). Samples were taken from plants showing typical symptoms of tospovirus infection, such as chlorotic and necrotic rings on the leaves and malformation and necrosis of the flowers. In Greece samples were tested by ELISA using polyclonal antibodies against the N protein of tomato spotted wilt virus, (TSWV (BR-01)) and impatiens necrotic spot tospovirus, (INSV (NL-07)). In Italy tests were performed using commercial TSWV and INSV kit antibodies (LOWE Biochemica) as well as monoclonal antibodies against a local isolate of TSWV from chicory. Positive samples were subsequently tested by mechanical inoculations, or electron microscopy. In Greece, the following species were found, mainly in greenhouses, to be infected with TSWV: *Anemone* sp., *Antirrhinum majus, Aralia japonica, Aster* sp., *Alstroemeria* sp., *Begonia* sp., *Beloporone guttata, Calendula officinalis, Callistephus chinensis, Celosia cristata, Coleus* sp., *Cineraria nana hybrida, Chrysanthemum* sp., *Dahlia hybrida, Dianthus sinensis, Dieffenbachia* sp., *Dimorphotheca sinuata, Fuchsia* sp., *Gazania* sp., *Geranium* sp., *Gerbera jamesonii, Impatiens* sp., *Iris* sp., *Mathiola incana, Ocimum basilicum, Pelargonium* sp., *Portulaca grandiflora, Petunia hybrida, Ranunculus* sp., *Saintpaulia ionantha, Salvia splendens, Solanum capsicastrum, Stephanotis floribunta, Tagetes erecta, Tropaeolum majus, Viola tricolor, Vinca rosea, Zantedeschia* sp., and *Zinia elegans*. None of the samples reacted with INSV. In Italy, *Gerbera jamesonii, Ranunculus* sp. and *Nerium oleander* were infected with TSWV and *Eustoma grandiflorum* was infected with INSV only or with both INSV and TSWV. The weed species *Erigeron* sp., *Sonchus oleraceus, Solanum nigrum* and *Amaranthus* sp., associated with ornamental and vegetable crops, were, in southern Italy, hosts of TSWV. In Greece the most prevalent weed hosts of TSWV in greenhouses were *Aster* sp.,

Senecio vulgaris, *Sonchus* sp., *Erigeron canadense* and *Stellaria media.* Thrips collected from infected plants, in both countries, were identified as *Frankliniella occidentalis* and *Thrips tabaci.*

OCCURRENCE OF AFRICAN CASSAVA MOSAIC VIRUS (ACMV) AND EAST AFRICAN CASSAVA MOSAIC VIRUS (EACMV) IN SOME CASSAVA CULTIVARS (*MANIHOT ESCULENTA*) AND WEEDS IN TOGO

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Cassava (*Manihot esculenta* Crantz) is the most important staple food in sub-saharan Africa , and the third largest source of carbohydrates in the world. The productivity of this crop in Togo is low as in most African countries. Many constraints could explain this poor preformance, amongst which pests and diseases are responsible for such serious losses. Cassava mosaic disease (CMD) is the most destructive one and it is economically the most important cassava disease. The disease is widespread and prevalent in most cassava growing areas in West Africa and elsewhere.

Recently it was proven that CMD is caused by three distinct whitefly-transmitted geminiviruses: African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and Indian cassava mosaic virus (ICMV), occurring in different geographical regions.

Since massive introductions of cassava planting material, horticultural and medicinal plants occur in Togo it is important to investigate the possible introduction into the country of these cassava geminiviruses which were previously restricted to some specific geographical regions. Therefore surveys were conducted in different prefectures in Togo where cassava is extensively grown, in order to identify the geminiviruses causing CMD. Fourty-eight foliar samples were collected from both cassava (39) and weeds (9), showing mosaic symptoms with leaf distortion, mottling, leaf curling and small leaves. Samples were analyzed using TAS-ELISA with polyclonal and monoclonal antibodies, and also PCR with specific primers. ACMV and EACMV were detected in both cassava cultivars and weeds. The following weeds from the family Euphorbiaceae reported by other authors as harbouring ACMV, were found infected by the two geminiviruses investigated during this study: *Jatropha curcas*, *J. multifida* and *Manihot glaziovii*. This is the first report of these viruses in *Cnidoscolus chayamansas* (Euphorbiaceae) an exotic vegetable. The presence of these viruses in weeds could play an important role in their spread and the maintenance of a continuous source of inoculum.

EPIDEMIOLOGY OF RASPBERRY BUSHY DWARF VIRUS IN RASPBERRY AND BLACKBERRY IN CZECH REPUBLIC

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Raspberry bushy dwarf virus (RBDV) was found for the first time in the Czech Republic in 1994. During 1995-96 the virus was monitored by ELISA (RBDV kit, Loewe Biochemica, Germany) in 16 cultivars of raspberry at five localities in Bohemia and Northern Moravia. Plants with RBDV-like symptoms (interveinal chlorosis, yellows of leaves) were selected preferentially in the tests. RBDV infected 22% of 88 plants collected in the farm fields. The virus was transmitted by mechanical inoculation from cvs. Bulharsky rubín, Gatineau, F-103 and from two unknown cvs. to *Chenopodium quinoa* plants.

In 1995-96 we tested 169 plants of 13 cvs. used for seed propagation at the Breeding Station at Velké Losiny; 43% of plants were infected with RBDV. Similarly, RBDV infected 17% of 59 plants tested from the germplasm collection at the Research and Breeding Institute of Pomology, Holovousy. BYDV was detected in 1 of 10 cultivars (Canby) derived from meristem tips. Therefore infected germplasm collections and propagation material seem to be the main source for extensive RBDV spread in raspberry in the Czech Republic.

No typical or uniform symptoms were observed on infected plants. Monitoring of 25 infected raspberry plants of 9 cultivars revealed interveinal yellowing and necrosis of leaf margins in cv. Canby, whereas other cultivars were symptomless. Most of the cultivars revealed severe symptoms of crumbly fruits and yield reduction in comparison with healthy control plants.

The virus was successfully transmitted by grafting from cvs. Bulharsky rubín, Meeker, Canby, Veten, Granat and Norna to virus-free plants of cv. Malling Jewel 4, kindly supplied by Dr. A.T. Jones (SCRI, Dundee, U.K.). This experiment provided evidence that the resistance-breaking isolate of RBDV occurs in the Czech Republic.

RBDV is transmitted by pollen. This fact raised the question, whether wild raspberries and blackberries forming dense canopies in Czech forests, could be a source of infection. Extensive indexing of 461 plants of wild raspberry taken from 68 localities revealed only 6.5% positive samples at 16 locations. In wild raspberry, from 118 plants tested at 34 localities only 6.7% were positive at 4 locations. Attempts to transmit the virus from wild plants to *C. quinoa* was not successful.

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EPIDEMIOLOGY OF GRAPEVINE LEAFROLL IN CENTRAL PO VALLEY (NORTHERN ITALY)

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Grapevine leafroll (LR) is a virus disease that remains widespread in central Po Valley (northern Italy) as in many others viticultural areas. It may cause severe damage with reductions of yield and fruit quality. Probably its frequent occurrence in old vineyards is mainly due to the use of virus-infected propagules. However, we recently observed natural spread of the disease even in vineyards planted with certified virus-free clones. Subsequent investigations showed that two

closteroviruses (GLRaV-1 and GLRaV-3), considered as causal agents of the disease, may be transmitted from grape to grape by three different species of scale insects (*Neopulvinaria innumerabilis* Rathvon, *Parthenolecanium corni* BouchÈ and *Pulvinaria vitis* Linnaeus). Further investigations are being carried out to verify whether those insects can transmit other closteroviruses associated with the disease.

EPIDEMIOLOGY AND MONITORING OF SOME PLANT VIRUSES IN UKRAINE

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Much attention has been given recently to research on the ecology and epidemiology of plant viruses. However, the problem of forecasting and preventing development of virus diseases can be solved only by taking account of the many factors influencing virus prevalence in different agro-coenoses. To major of such factors it is possible to relate biology of a virus, type of a cultural plant in agrocenosis, climatic conditions, structure both quantity of weeds - reservoirs and insects - vectors of carry of a virus, anthropogenous pressure on bio- or agrosystems. In this connection the purpose of work was to study the dynamic prevalence of some viruses of plants in various ecological regions of Ukraine and to find out interrelations between phytoviruses epidemiology and action of the factors of external environment.

During 1994-1997 10 agrocenosis and 2 biocenosis in each of 4 areas of Ukraine were surveyed. Coenoses were surveyed visually, then the samples of crop plants, weeds and soil were taken for test (not less than 3 replicates). The tests were analyzed in IFA (DAS-, TAS-ELISA) on standard techniques using antibodies against WSMV, BYDV, BSMV, BMV, BMYV, TMV, PVX, PVY and other viruses; the preparation were supervised on electron microscopy. Pollution of soil was investigated on atom-adsorption spectrometre. Statistical processing of results and creation of a database were carried out using a IBM AT PC 486 DX-66 and modernized program ACCESS (Microsoft Office).

The results of research have shown non-uniformity of distribution of virus infections of plants in various ecological regions of Ukraine. In spite of the fact that all regions (except for one, control) were in identical climatic and geolandscape conditions, the presence of antigenes of the majority of viruses considerably prevail in ecologically unsuccessful regions exposed to significant anthropogeneous pressure. It is characteristic not only for agrocoenoses, but also for “wild” (uncultivated) coenoses and can be explained as negative influence of only for agrocoenosis, but stability of plants to viruses, and/or increased infectivity of a virus particle (because of mutation). The observed dynamics of presence of antigenes of some viruses in agrocoenoses and connection of concentration of a virus in agrocoenoses with crop-rotation and weather conditions. The presence of antigen of viruses not only in cultural plants, but also in accompanying weeds and soil is shown. These data can be used for forecasting and preventive maintenance of virus diseases developed of plants in concrete agrocenosis. A large amount of data has allowed to create a databank on distribution of various viruses in various agrocoenoses and its dynamics. On the basis of this databank the control system of databases allowing to predict probably of development of that or other virus diseases is created.

A CEREAL DISEASE CAUSED BY MITE-TRANSMITTED VIRUSES

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Wheat streak mosaic virus (WSMV), a member of the *Poytviridae*, infects winter wheat in Israel, especially the beard wheat *(Triticum durum).* Recently we also found the high plains virus (HPV) in seed-production plots of maize. The local vector in Israel has not yet been identified. Information from other countries points to the wheat curl mite *(Aceria losichella)* as the vector. This species has the potential of transmitting both viruses simultaneously to wheat and corn with potentially devastating effects on growth and yield, as in Kansas and Colorado. WSMV was not detected in corn in Israel though it was found in all wheat-growing areas. Most corn grown in Israel is sweet corn that is not affected by HPV and therefore the outbreaks of these virus epidemics were restricted due to the limited area of susceptible corn grown. The expanding corn seed production may be susceptible to HPV. Furthermore almost all the corn lines grown in Israel are susceptible to the aphid-transmitted maize dwarf mosaic potyvirus (MDMV). Therefore, the devastating possibility exists of infection with the two different viruses transmitted by different vectors. Although the wheat curl mite has not yet been identified in Israel, the natural spread of WSMV and HPV indicate that an arthropod vector exists. *Graminaceous* weeds which harbour the various cereal viruses (Johnson grass and Yellow cotton tail) are common at the edges of irrigated plots and therefore virus reservoirs for spread by the vectors are also available. Since all the component factors occur in Israel, it is interesting to consider why the vector-virus-host interaction has not yet resulted in mixed infections. The long dry season may present a barrier to transmission of viruses from wheat to corn and *vice versa*, thus preventing the build-up of mixed infections.

IDENTIFICATION AND DETECTION OF VIRUSES OF ZANTEDESCHIA

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About eight viruses are known to occur in *Zantedeschia* of which dasheen mosaic potyvirus (DsMV) is the most prevalent and widespread (Zettler and Hartman, 1987. Plant Disease 71: 958-963).

Diseased plants of various *Zantedeschia* species and hybrids were tested in DAS-ELISA with DsMV-antiserum and in ACP-ELISA with two potyvirus-specific monoclonal antibodies. Although diseased plants of *Z. albomaculata* and some hybrids appeared to be infected with a potyvirus, no reaction was obtained with the DsMV-antiserum. Coat protein fragments of this potyvirus and of DsMV from other *Zantedeschia* hybrids were amplified in RT-PCR with potyvirus-specific primers. The fragments obtained after restriction enzyme digestion were identical for both potyviruses indicating that these are strains of the same virus.

Virus-free stocks of *Z. albomaculata* can be obtained from seed. For hybrids meristem culture is needed to obtain virus-free material. Plants grown from meristems are tested with the potyvirus-specific monoclonal antibodies. Most reliable results are obtained by testing leaf material. The virus cannot be tested reliably in the tubers of various cultivars during the storage period. Virus concentrations are higher in the side parts than at the top of the tubers.

CHARACTERIZATION OF AN UNUSUAL POTY-LIKE VIRUS INFECTING TOMATOES IN THE CENTRAL SUDAN

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Surveys for virus diseases infecting pepper and tomato crops were carried out in central Sudan from 1994-1997. Samples were collected from different regions and during different growing seasons. The preliminary results revealed besides tomato mosaic virus (ToMV), cucumber mosaic virus (CMV), potato virus Y (PVY) and tomato yellow leaf curl virus (TYLC), the presence of unusual symptoms related to an unreported virus of tomato. The symptoms comprised intense interveinal yellowing, deformation and crinkling of the leaves, bushy top appearance of the whole plant and fruit malformation. The virus is transmitted efficiently by mechanical inoculation and also by contact under experimental conditions.

Hosts of the previously unreported virus were confined to the *Solanacea* of the twelve families tested. The virus causes vein-clearing symptoms in *Lycopersicon esculentum*, *Nicotiana tabacum*, *Datura stramonium*, *Petunia hybrida*, *Physalis* spp. and symptomless infection of *Solanum dubium* and *S. nigrum* but does not infect pepper, potato or eggplant. The infectivity of crude sap withstood in vitro-aging for *c*. 30-days, has a thermal inactivation point between 65°-70°C, and

dilution end point 10^{-3}-10^{-4}. DAS-ELISA tests with crude extracts showed no serological relationship against antisera of ToMV, potato virus M, potato virus S or potato virus X.

Electron microscopy examinations in negatively stained dip preparations showed long filamentous virus-like particles. Virus particles as well as cytoplasmic cylindrical inclusion (pin-wheels) laminated aggregate and scroll were also observed in ultrathin sections prepared from tomato. The particles measured 750-950 nm in length. The mean length based on 120 particles was 850 nm. DAS-ELISA tests with crude extract against antisera of PVY, pepper veinal mottle virus and tobacco etch virus showed noreaction. An antiserum against one of the selected strains has been produced. Seed transmission in tomato and insect virus transmission studies are in progress. These preliminary results indicate that the tentatively named "Tomato bushy top" virus may be a poty-like virus even though it did not resemble any of the potyviruses previously reported.

EPIDEMIOLOGY OF A MAIZE VIRUS DISEASE TRANSMITTED BY A PLANTHOPPER IN THE MEDITERRANEAN AREA

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Since 1980 we have found stunted plants showing typical virus-like symptoms in nurseries and in some corn seed production fields in southern France and northern Spain. Yellow streaks of different sizes appeared on the leaves. These bands rapidly coalesced into long stripes while the leaves turned yellow then red. Leaf dip preparations revealed numerous bacilliform particles (220-260 x 60 nm). Transmission trials with the planthopper *Laodelphax striatellus* were successful.

An epidemiological study was conducted. Symptoms development was recorded on six maize plots sown every 15 days from the beginning of May; 2-week-old bait plants were also left in the field for 10-day periods. Sticky yellow traps and sticky fishing line traps were used to monitor planthopper populations. Thus the pattern of the disease in the field was determined. Symptoms were observed from July 6 th until October 17 th. The percentage of trap plants that became contaminated varied from 16 to 51. The planthoppers caught were determined, and *L. striatellus* was the most frequent (more than 60 %). The planthopper population was largest from June 16th till August 16 th. Observations of the surrounding weeds revealed the same virus in *Cynodon dactylon*. The virus differed from maize mosaic but seemed to be related or identical to cynodon chlorotic streak virus described in Morocco by Lockhard in 1985.

FORECASTING POTATO VIRUS Y USING SUCTION TRAPS

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In Sweden, aphid-borne potato virus Y (PVY) is more frequent in potato than potato leaf roll virus (PLRV), which is also spread by aphids. This can probably be explained by the lower abundance of *Myzus persicae* in potato fields compared with aphid species transmitting PVY. However, the degree of spread of PVY varies greatly between regions and years. Although PVY can be transmitted by aphid species that feed preferentially on potato, other species that do not colonize potato seem to be far more important, e.g. *Rhopalosiphum padi, Brachycaudus helichrysi, Acyrthosiphon pisum* and *Phorodon humuli*.

During the last decade there has been increasing interest in developing methods for PVY forecasting. The main variables used include the number of alates and their vector efficiency, the time of aphid migration in relation to plant age, and the availability of virus sources. We investigated the relationship between suction trap catches of aphids and proportion of PVY-infected progeny tubers, and considered the importance of mature plant resistance and proportion of virus sources.

Aphid data from five suction traps in Sweden during 1985-1992 were collected. Eight aphid species were identified: *A. pisum, Aphis fabae* Gr., *Aphis nasturtii, A. frangulae, Brevicoryne brassicae, Metopolophium dirhodum, M. persicae, R. padi* and *Sitobion avenae*. All other less common species were assigned to "Other aphid species". In each region data concerning cultivar, proportion of PVY-diseased potato plants acting as virus sources (assessed by field inspection) and proportion of PVY-infected progeny tubers (determined by post-harvest testing) were collected from *c*. 600 potato fields within 10 kilometres of a suction trap.

Suction trap catches varied greatly between years and regions. *R. padi* was the most common aphid species. The relationship between the total number of winged aphids and proportion of PVY-infected progeny tubers was quite close. The relationship was even better when taking into account the effect of mature plant resistance, the main vectors and proportion of virus sources (R^2= 0.81). When analysing relationships each successive week there was a close correlation when taking into account the catches of main vectors until the end of June and the proportion of virus sources (R^2=0.85).

COMPARISON OF APHID SAMPLING METHODS IN CITRUS

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Citrus tristeza virus causes serious damage to citrus around the world and is transmitted by aphids, mainly *Aphis gossypii* in the Mediterranean countries. In Spain, *Aphis spiraecola* and *Aphis gossypii* are at present the most abundant aphid species on citrus. Traditionally, yellow water traps

have been used to establish their proportions, but a few years ago it was discovered that these traps attracted *A. spiraecola* more strongly than other species.

In this work we have compared, over four years, the following methods of aphid assessment in several orange and clementine orchards of the Valencian Country: water traps (yellow, green and a mixture of yellow and green), sticky fishing-line traps, sticky tree method, and sampling of colonies on leaves. The relative proportion of *A. spiraecola* and *A. gossypii* in captures of colonies on orange leaves is equivalent to that of the yellow water trap if the orchard is planted with orange and clementine mixed, or to that of water trap with yellow and green mixed if the orchard is only planted with orange trees. In these cases, water traps could be used instead of sampling aphid colonies on orange leaves.

EPIDEMIOLOGY OF ZUCCHINI YELLOW MOSAIC VIRUS IN THE NETHERLANDS

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Since the early eighties zucchini yellow mosaic virus (ZYMV) has been found occasionally in summer squash (*Curcurbita pepo*) and cucumber (*Cucumis sativus*) in the Netherlands. The infections occurred both in field and greenhouse crops and, with one exception, were first noticed in late summer.

To eradicate the virus it appeared sufficient to destroy the infected plants. New infections were never observed at or near the same locations. This may be explained by the absence of host plants that would enable the virus to survive during the winter outdoors.

Since the introduction of ZYMV via imports of infected plants and seeds is not considered feasible, most probably the virus was introduced by viruliferous aphids transported by low-level jet winds from the Mediterranean region. This means of long-range spread for non-persistently transmitted plant viruses has been suggested previously (Zeyen & Berger, 1990). It may also be applicable for ZYMV, since it was found that aphids may remain viruliferous for over 30 hours if they are not allowed to probe (Fereres *et al*., 1992).

TRANSMISSION OF ZUCCHINI YELLOW MOSAIC POTYVIRUS BY DIFFERENT APHID SPECIES NOT COLONIZING CUCURBITS

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Zucchini yellow mosaic virus (ZYMV) is a member of the family *Potyviridae*, and transmitted non-persistently by aphids. In field experiments carried out in northern Greece (Vassilica), total number of aphids captured by Moericke yellow traps, in experimental plots was correlated to ZYMV spread. Several aphid species were reared on suitable hosts and tested for virus transmissibility from and to zucchini under laboratory conditions. *Myzus persicae*, a known vector of ZYMV, was found to be the most efficient one, transmitting five isolates of ZYMV with similar efficiency (38 - 45 %). Several aphid species, not known previously as ZYMV vectors, were found to transmit: *Aphis craccae, Aphis fabae, Aphis nerii, Aulacorthum solani, Brachycaudus cardui, Brevicoryne brassicae, Hyalopterus pruni, Hyperomyzus lactucae, Macrosiphoniella sanborni, Macrosiphum rosae, Metopolophium dirhodum, Myzus cerasi, Rhopalosiphum padi, Rhopalosiphum maidis, Semiaphis dauci*, and *Sipha (Rungsia) maydis*. Their transmission efficiency was low (0.07 - 3.0% on a single aphid basis). Three species, *Hayhurstia atriplicis*, *Myzus ascalonicus* and *Sitobion avenae* did not transmit the virus. Six of the reported new vectors were assayed in arena tests to estimate propensity. In all cases, except *Aphis nerii*, propensity was higher than efficiency. The most abundant species among the new vectors were *Rhopalosiphum padi*, *Hyalopterus pruni*, and *Metopolophium dirhodum* as determined by Rothamsted type suction trap captures in four regions (Thessaloniki, Velestino, Kopaida, Pirgos) over two years. Total number of new and already known vectors ranged between 40 and 80% of the total number of aphids captured, depending on region and year. Of the known vectors the most abundant was *Aphis gossypii*, whereas *Myzus persicae* appeared in all regions in low populations. Most of the new vectors tended to appear early in the growing season (March-April), thus presumably being responsible for early infections. The importance of the new vectors in ZYMV epidemiology is discussed.

TRANSMISSION OF RICE YELLOW MOTTLE VIRUS BY THE CHRYSOMELID BEETLE, *TRICHISPA SERICEA*

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The beetles that are known to transmit rice yellow mottle mottle virus (RYMV) are in the family Chrysomelidae, commonly known as leaf beetles. This family contains thousands of different species having diverse body shapes. One of the most distinctive species is a rice-feeding beetle, *Trichispa sericea* (Bakker 1974). The adults scrape the leaf lamina clean between the veins of the rice leaf. Eggs are normally deposited at the tips of the rice leaves and the larvae feed from the mesophyll tissues between the upper and lower epidermal surfaces. The tunnelling of the larvae produces long, translucent lines in the leaf. One beetle and 3-4 leaf-mining larvae can severely damage seedling plants. Despite its identification as a vector of RYMV by Bakker in Kenya, there is little evidence to support its role as a major vector in Tanzania. In 1997, a survey of rice fields showed a high incidence of RYMV in the unbunded, shallow flooded areas of the Kilombero valley. The presence of RYMV in this region was confirmed by serological tests, but neither the beetle vectors nor evidence of beetle feeding damage was found. In the bunded fields around Lake Victoria, RYMV was only observed in small patches within or at the edge of fields. In this region, however, low numbers(<1 adult per 10 fields)of *T. sericea* were collected from bunds surrounding fields containing plants 20-45 days old. No beetles were collected from or around fields containing

plants 45 days old. Laboratory tests confirm that *T. sericea* is an inefficient vector of RYMV. Insects allowed to feed on RYMV-infected plants for 72hrs were transferred immediately to healthy seedlings (10 days after sowing) for 24hrs. Although extensive feeding damage was observed only low transmission rates (7-15%) were recorded. Numerous other chrysomelid beetle species occur in and around rice fields, but these rarely cause major feeding damage to rice crops. As these preliminary results indicate that there is not necessarily a correlation between feeding damage and transmission efficiency, it is possible that one or more of these species may be a more efficient vector of RYMV than *T. sericea*.

REARING OF *CEROTOMA ARCUATA* OLIV. (COLEOPTERA; CHRYSOMELIDAE) AND ITS EFFICIENCY IN TRANSMITTING AN ISOLATE OF COWPEA SEVERE MOSAIC VIRUS

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Cerotoma arcuata is vector of cowpea severe mosaic *Comovirus* (CpSMV) and of several leguminous viruses in Brazil. An isolate designed CpSMV-SP was found on *Vigna luteola* from the coastal area of the São Paulo State. To gain a better understanding of the virus transmission process, the following interactions were studied: virus/host, vector/host and vector/virus/host. A rearing technique for *C. arcuata* was adapted to laboratory conditions. Larvae were fed with pre-germinated bean seeds and adults were fed on bean seedlings. To determine the sex ratio providing the best oviposition and longevity, field-collected beetle were tested keeping different sex ratios (2:1, 1:1 and with no defined ratio). Two substrates were evaluated: a) organic soil and b) vermiculite+organic soil+sand. Colonies with a 2:1 sex ratio showed higher longevity and oviposition. Better results were obtained by using substrate b; it gave higher viability and shorter total life-cycle of the beetle. The transmission rates by adults were 47, 50 and 70% on bean and by 1st instar larvae were 40% on bean and 10% on cowpea. Transmission by other arthropods (aphids, whitefly, thrips and mites) and by other coleoptera species failed. Field-collected and laboratory-reared beetles can transmit CpSMV-SP, but transmission rates vary with host and virus source. Females were more efficient vectors than males. Males were able to retain virus and transmit for at least 14 days and females for up to 10 days. *C. arcuata* showed a greater feeding preference on *Glycine max* and *G. javanica* than on *V. unguiculata* and *Phaseolus vulgaris*. *C. arcuata* fed preferentially on healthy rather than on virus-infected soybean plants. Only species of 2 of 5 families tested were infected mechanically: Chenopodiaceae (*Chenopodium amaranticolor*) and Leguminosae (*Glycine javanica*, *G. max*, *Macroptilium lathyroides*, *P. lunatus*, *P. vulgaris*, *V. luteola*, *V. mungo* and *V. unguiculata*). The CpSMV-SP differs from the type-strain by infecting many bean and soybean cultivars; cowpea was less susceptible. The high susceptibility to the virus, the occurrence of many perennial hosts and the presence of the vector may cause a problem for leguminous crops.

INCIDENCE AND DISPERSAL OF BEAN COMMON MOSAIC AND BEAN COMMON NECROTIC MOSAIC POTYVIRUSES IN BEAN FIELDS OF CASTILLA PLAINS OF SPAIN

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Bean common mosaic and bean common mosaic necrotic potyviruses are the main pathogens of beans in Spain (Sáiz *et al*., 1995). The dispersion and dissemination of these viruses were studied in three different ecosystems of the Castilla plains: i) La Bañeza in the León province, a zone having a long tradition of bean cultivation ii) Serrada in the province of Valladolid, where cultivation is limited and iii) Barco de Avila in the province of Avila, valley where beans have a denomination of origin. Analysis by ELISA and RT-PCR of the seeds before the establishment of the crop showed an incidence of 1 to 10% infection depending on the varieties used. To study the dispersion of the virus in relation to the time of cultivation, we used an experimental design of randomly sampled plants in the bean fields which were tested by ELISA every 15 days. Disease incidence reached 100 % in León and Valladolid fields; though their effect on production was determined by the time of their infection, and total loss of crop occurred only in plants infected as seed or during early growth. In the fields of the Barco de Avila, despite seed-borne inoculum, the viruses did not spread, probably due to the lack of aphids flight, as influenced by the geographical characteristics of this zone. The evaluation of the diseases during growth and their effect on production will be discussed.

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EPIDEMIOLOGY OF CHICKPEA CHLOROTIC DWARF VIRUS IN FABA BEAN AND CHICKPEA IN SUDAN

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Faba bean and chickpea constitute the major food legumes grown and consumed throughout Sudan. The main production area is the Northern provinces of the country where the relatively cool winters provide optimal growth and yield conditions. During the past few years attempts were made to expand their production towards the south where much more fertile lands are available. However, crops in the new area suffered from a severe, previously unknown, yellowing disease that threatened to halt production. Later, the disease became widespread throughout all production areas, especially in the southern parts. The causal virus was identified as chickpea chlorotic dwarf virus (CCDV) in 1996, and epidemiological investigations were initiated thereafter. Surveys in all production areas in Sudan revealed that CCDV is the most important and most widely distributed virus causing legume yellowing in the Sudan. The virus was transmitted by the leafhopper *Neolimnus aegyptiacus* which was very prevalent in legume fields throughout the year. This leafhopper vector transmitted CCDV to a wide variety of wild and cultivated legume species. Studies of the spatial patterns of virus spread in the field indicated random spread from primary infections and subsequent virus spread did not seem to correlate with wind direction. However, field experiment also revealed that the highest virus incidence correlated strongly with early sowing date. ln chickpea, infection with CCDV strongly affected all yield parameters including both qualitative and quantitative aspects. Yield losses may therefore reach 75% compared with healthy plants.

INCIDENCE AND SPREAD OF LILY SYMPTOMLESS AND LILY MOTTLE VIRUSES IN LILIES IN THE NETHERLANDS

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The incidence of lily symptomless virus (LSV; non-persistently transmissible by aphids) and lily mottle virus (LMOV; formerly named tulip breaking virus; non-persistently transmissible by aphids) should be maintained at the lowest possible level. Various factors affect the incidence of both viruses. overall, efforts were successful to obtain virus-tested material of all cultivars by tissue culture procedures from the early 1970s onwards in order to bulk-up lots of bulbs for further growth and ultimately to produce cut flowers. Nevertheless a zero virus incidence is not attainable as a result of factors indicated. Symptoms of both viruses are different in all cultivars with occasionally predominantly mild or masked symptoms. This precludes the efficient roguing of diseased plants in the field. Symptoms may show differently under field and greenhouse conditions and occasionally they are more harmful in the greenhouse than suggested by the naming of a virus like LSV. This qualitative effect together with the reduction of bulb yields by both viruses emphasises the need for control.

Tests for LSV and LmoV by DAS-ELISA are performed routinely on hundreds of thousands of bulb and/or leaf samples by the Flower Bulb Inspection Service. The serological test largely replaced visual disease control based on roguing plants in the field. In general the viruses spread rapidly in field crops as effectively affected by the rate of tolerance to virus infection possibly still too high occasionally. The control of virus spread by mixtures of mineral oil and pyrethroid insecticides will be essential to maintain infection rates of the lots at or below an acceptable level. The propagation rates of virus-tested material by tissue culture procedures and those by the scaling of bulbs under the general culture conditions enable growers to replace fairly rapidly badly infected lots by adopting qualitatively improved material. Experimental data on all factors affecting the virus health status of lilies will be presented and discussed.

INCIDENCE AND SPREAD OF THRIPS -BORNE TOMATO SPOTTED WILT VIRUS IN DAHLIA IN THE NETHERLANDS

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Tomato spotted wilt virus (TSWV) has long been known to occur in dahlia. In the early 1990s the virus incidence in the dahlia crop (*c.*430 ha) was at a very high and unacceptable level. This created the need to study the problem experimentally. Infection occasionally caused symptoms but largely did not in a wide range of cultivars. The failure to detect diseased plants efficiently in field crops could be overcome effectively by testing tubers by ELISA. Virus detection in tubers was possible after long storage (9°C) at the end of the year or later before replanting the tubers in late January so as to harvest cuttings in the following months to be used for the production of tubers in the field from late May till November. In laboratory tests two thrips species, i.e. *Thrips tabaci* and *Frankliniella intonsa* caught from field-grown dahlias were found to transmit TSWV. In the field *B.*

tabaci appeared to be the most abundant species. TSWT spread in the field but infection pressure was variable in consecutive years, e.g., 1992: 3.0%, 1993: 2.9% (exp. 1) and 16.4% (exp. 2), 1994: 2.3%, and 1995: 10.8%. Virus spread was strongly dependent on the distance from infected plants acting as a virus source near to the plants to be infected, which is to be reckoned in a few or more metres. Data on the mainly short-distance spread of TSAT will be presented. The data on the pattern and impact of short-distance spread in dahlia which can be propagated rapidly by cuttings (1 5-40/tuber) and the efficient testing of tubers by ELISA, imply that the chemical control of TSVN spread by insecticides may be omitted if mother-TSWW-tested lots are planted at suitable distance from other dahlia material grown for commercial production.

STUDIES ON YIELD AND VIRUS INFECTION OF A GARLIC COLLECTION

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In the last three years, two trials were carried out in Matera area (Basilicata region, southern Italy) to evaluate yield and its components (plant, leaf, bulb and bulblet size) as well as virological state of a garlic entry collection of Italian and foreign (France, Spain, Albany, Greece, Chile and Turkey) origin. A randomized block design with three replicates was followed. Planting times were October 23, 1996 and January 21, 1997, for the first experiment, and December 15, 1997, for the second one. Bulblets of each entry were planted 7-8 cm deep, with 40 cm between rows and 10 cm within row in plots of 6 m^2 each. Yield traits were determined at harvest time, i.e. on June and July 1997 and 1998. Viral infections were assayed by DAS-ELISA using sap extracted from leaf tissues of single garlic plants *c*. 40 days before harvest.

Results obtained showed a great variability in yield features among the garlic entries studied and the variable but uniformly high incidence of two Potyviruses, i.e. onion yellow dwarf (OYDV) and/or leek yellow stripe (LYSV). Bulb average yield varied from a minimum of 1.7 t/ha, for 'Germidour' violet garlic to 10.4 t/ha for 'Irsina'. In the first trial, all garlic entries except 'Di Pescia', 'Ail Cristo', 'Ail Rose Printanor', 'Ail Printanor', 'Ail Rose D. Avvergne' and 'Castigliano' gave higher bulb yield when planted in October 1996 than in January 1997. In contrast, the above six entries grew better in the second planting and produced a yield increase equal or superior to 1 bulb t/ha.

OYDV and LYSV were detected in high percentage (60-100%) and often in mixed infection in the great majority of garlic entries and in medium to low percentage (50-13%) in some of them. In 1997, OYDV was not found in any plants of "Irsina" and "Tricarico" and "Gravina 1" and "Marsicovetere 2" were not infected by LYSV. The same garlic entries were infected by single viruses with incidences varying from 30 to 80% in 1998.

SURVIVAL OF TOMATO SPOTTED WILT VIRUS UNDER CONTINENTAL CLIMATIC CONDITIONS

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Tomato spotted wilt *tospovirus* (TSWV) has long been known in Central- Eastern- and Southern Europe. However, the frequency of epidemics has been increasing. In the last five years epidemics were observed in Hungarian tobacco fields as well as in pepper and tomato crops in greenhouses, causing serious yield losses. The aim of our study was to investigate the epidemiology of TSWV under a continental climate, in which no cultivated hosts are available for overwintering.

Only *Thysanoptera* species transmit *tospoviruses*. Larvae of vector species can acquire the virus and they can retain it as adults until death. Population dynamics of *Thrips tabaci* and *Frankliniella occidentalis* were monitored by yellow and blue sticky traps, respectively. In compliance with the host range, phenology and hibernation, the theletokous *T. tabaci* populations have a significant role in the epidemics of TSWV in tobacco fields. TSWV could persist in winter only in the infected *T tabaci* adults and in biennial and perennial herbaceous host plants that act as reservoirs. Females spend the winter in litter and parched grass and on green parts of overwintering plants, e.g. *Lamium purpureum, Stellaria media* and *Trifolium repens* in ruderal fields. Adults could migrate in spring to nursery-beds or to the tobacco fields immediately following transplanting. Although the individual number of hibernating insects is relative low, nevertheless they are able to cause considerable infection especially of the young seedlings in nurseries which are very susceptible to infection during this period.

F. occidentalis is known as the most active vector of TSWV. It was introduced to Europe in 1985 and it spread in the following years in greenhouse crops. It cannot hibernate in fields under temperate climatic conditions. It can survive during the growing season in the field in the flowers of different plants. Some of them, e.g. *Chenopodium album, Convolvulus arvensis, Galinsoga parviflora, Melilotus officinalis, Stellaria media* and *Trifolium repens* are hosts of TSWV. Therefore it serves as a vector and is able to transmit the virus from cultivated plants to weeds and *vice versa*. It has a significant role in the spread of TSWV in greenhouses.

Recently *F. intonsa* has been also recorded as TSWV vector. It occurs frequently in the flowers of different wild plants and weeds, e.g. *Asclepias syriaca, Galium verum, Lathyrus tuberosus, Medicago sativa, Melilotus officinalis, Trifolium pratense, Vicia sativa*, and in the flowers of ornamental plants, but seldom in the flowers of tobacco, pepper or tomato. Its role in the spread of TSWV requires further study.

LONGEVITY OF HIBISCUS LATENT RINGSPOT NEPOVIRUS IN SEED OF KENAF

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Kenaf (*Hibiscus cannabinus* L.) seed may contain hibiscus latent ringspot nepovirus (HLRSV) in the embryo. To investigate longevity of HLSV in seed stocks of cv G4 purchased from Australia in 1993 were tested at different times. Seeds were stored in the dark at 6±1°C. Each whole

seed was assayed individually for HLRSV using an indirect protein A sandwich-enzyme linked immunosorbent assay method (PAS-ELISA). Individual seeds were surface-sterilized in tri-sodium phosphate 10% (Na_3PO_4) for 30 min and rinsed thrice with distilled water. Following imbibition in water for 3h, individual seeds were ground with a mortar and a pestle in five volumes of PBS containing 0.05% Tween 20, 2% polyvinylpyrrolidone (MW 24,000), 0.13% sodium sulfite and 0.2% ovalbumin. PAS-ELISA was used to assay virus infection of whole seed, and any external contamination of the seed was determined by assaying the first and third water washes.

Seeds of the same lot were surface-sterilized in tri-sodium phosphate 10% (Na_3PO_4) for 15 min, rinsed with distilled water and sown in sterilized soil (1:1 turf:sand) in a glasshouse at a temperature of 21$\pm$1°C and a 16h photo-period. Stringent isolation, confinement, handling, and insect control measures were taken to prevent outside contamination during the study. Each plant was grown singly and assayed for HLRSV at the 4-5 leaf growth stage using PAS-ELISA. For each plantlet assayed, portions of the cotyledon and of the youngest emerged leaf were pulverized with a mortar and pestle in ten volumes of the extraction buffer as described previously.

Results of the ELISA of whole seed revealed 51% (178/350) of kenaf seed infected in 1994, while in 1998 this decreased to 39% (33/ 90). The progeny seedlings assayed in 1994 showed a seed transmission rate of *c*. 26% (125/480). Rates of 25% (33 /129), 26 % (27/110) and 23% (22/98) were recorded. These data demonstrated longevity of HLRSV in seed for at least four years during which time it remains almost unchanged while the rate of infected progeny seedling decreases.

The results confirm the possible role of HLRSV seed transmission in virus perpetuation and dissemination. As kenaf is being investigated throughout the world as a potential new source for cellulose, and has become important in several countries as an industrial crop, the virus may easily be spread in germplasm and crop seed.

THE RECENT DECREASE IN VIRUS YELLOWS OF SUGAR BEET IN MIDDLE GERMANY

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The incidence of sugar beet plants with the beet mild yellowing luteovirus, the beet yellows closterovirus and the beet mosaic potyvirus was very high in beet fields of the former German Democratic Republic, especially in the districts of Magdeburg, Halle and Leipzig. After the unification of Germany the situation changed fundamentally. The incidence of beet viruses was reduced from a mean of nearly 50% in the years from 1980 to 1990 to *c*. 2% from 1991 to 1998.

Changes in the flight activity of the most important vector species *Myzus persicae* and *Aphis fabae* were not responsible for the decrease. Comparisons of the flight activity of aphids, recorded by a Rothamsted-suction trap at Aschersleben showed no differences since 1985. Although there are relatively great variations between the years in the total number of the trapped aphids, general changes between the periods 1985 to 1990 and 1991 to 1998 were not detectable. Furthermore, no trends for later or earlier occurrence of the first trapped aphids were observed.

One of the main reasons for the high virus incidence before 1991 was the presence of fields with overwintering plants retained for seed production in this region and the relatively short distances between sugar beet fields grown for sugar and seed production. The vector species *M. persicae* and *A. fabae* could transmit in the spring the viruses in virus reservoirs in the overwintering fields to the spring-sown fields used for sugar production. From there the virus was transmitted back to the autumn-sown seed production fields.

Following unification the agricultural situation changed. Seed of *B. vulgaris* is now obtained from countries of southern Europe. We believe that this is the main reason for the drastically reduced occurrence of yellows viruses. Furthermore, the area of sugar beet production has been reduced and better agronomic conditions and new varieties also increased the yields.

EPIDEMIC OUTBREAK OF TOMATO SPOTTED WILT VIRUS IN POTATO FIELDS IN THE CENTRAL REGION OF PORTUGAL

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Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus* (*Bunyaviridae*) and has a wide host range including more than 900 plant species of more than 60 families.

In Portugal, TSWV was identified for the first time in 1990 (Louro, 1991) and during the last years became an increasing problem in vegetable crops including tomato, pepper and lettuce and also in ornamental plants (Louro, 1996). In autumn 1997 in a restricted area named Península de Setúbal in the Central Region of Portugal, TSWV was detected in potato crops growing in open fields where a TSWV epidemic in tomato had been recorded (Serra, Weidemann and Vaz, 1998). The present work reports a severe outbreak of TSWV in spring and autumn potato crops during 1998 in the same area. The plants showed necrotic ringspots on leaves as well as top necrosis and tubers developed external and internal necrotic rings.

In order to understand these epidemics a 3- year TSWV survey program was begun in the Central Region of Portugal in 1998, to evaluate the alternative hosts present throughout the year as sources of TSWV and to define control measures to avoid the spread of the virus. The fields were sampled for several weed species present during the winter before crop plantings, weeds associated with potato and tomato crops as well as potato and tomato plants during the growing season. The the thrips vector *Frankliniella occidentalis* Pergande was recorded since the beginning of the year, due to relative warm winter conditions and increased in numbers from spring to summer. On the basis of TSWV infection frequency, the most important weed species that might be reservoirs of this virus during the year were *Arctotheca calendula* (L.) Levyns, *Solanum nigrum* L., *Datura stramonium* L. and *Sonchus oleraceus* L.. The observations in potato fields from several farms suggest that potato plants acquire TSWV from infected thrips coming from different sources such as overwintering infected larvae in soil, infected winter weeds and infected plants from neighbouring glasshouses. For the second potato crop (summer crop), tomato is an intercrop that plays an important role as source of TSWV and the vector. Cultural practices also influence the incidence of TSWV in potato fields.

VECTOR DEPENDENCY BY VIRUSES: INSIGHT INTO VIRUS-MEDIATED CHANGES IN HOST PLANTS THAT AFFECT VECTOR PERFORMANCE

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Although the potential advantages to vectors that transmit disease agents was recognized as early as 1951 by J. S. Kennedy, relatively limited attention has been devoted to the evolutionary and epidemiological consequences of interactions among viruses, vectors and plants. A few studies have demonstrated either beneficial, neutral or detrimental effects on vectors according to the particular pathosystem considered. A question raised by the variable outcomes of these studies is whether there is any theoretical basis for explaining why effects on vectors vary, and if there is any pattern to how they vary. The concept of ‘vector dependency’ by viruses is proposed as a means of better

understanding the outcomes of interactions among viruses, vectors and plants from the standpoint of the vector species involved. Because viruses vary considerably in their relations with vectors from complete dependence upon a principal vector species for dispersion to complete independence, i.e. a non-vectored virus, the relative dependency of a virus on one or more vector species may be of importance hypothetically to understand outcomes from a vector-performance perspective. The fitness of a vector is closely linked to host-plant quality, which in turn is influenced by virus-mediated changes in host-plant biochemistry. Therefore, the vector-dependency hypothesis posits that viruses that are more dependent upon vectors will indirectly benefit those vectors through favorable changes in the quality of their host plants. As Kennedy stated it: *"An agent which, through the disease it causes, actually multiplies its own vectors, has an obvious evolutionary advantage over one that does not."* In contrast, viruses having less-dependent relations with vectors do not risk as much evolutionarily if the diseases they cause are not beneficial to potential vector species. Tests of the vector-dependency hypothesis require examination of vector-performance studies to determine if beneficial outcomes are aligned with viruses that are satisfactorily 'vector dependent'. Results from aphid and whitefly performance studies will be presented as a test of this hypothesis.

CUCUMBER MOSAIC VIRUS IN ALTERNATIVE PULSE AND ANNUAL PASTURE LEGUMES: SUSCEPTIBILITY AND SEED TRANSMISSION

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Cucumber mosaic virus (CMV) causes diseases in several pulses and pasture legumes in which it is seed-borne, such as narrow-leafed lupin (*Lupinus angustifolius*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), subterranean clover (*Trifolium subterraneum*) and annual medic (*Medicago* spp.). In southern Australia narrow-leafed lupin (*L. angustifolius*) is the most widely grown pulse crop, but alternative pulses are being evaluated that grow well on fine-textured, neutral soils to which lupins are poorly adapted. In addition, traditional pastures based on subterranean clover and annual medics are being extended by exploiting alternative annual pasture legume species. The susceptibility and sensitivity of alternative pulse and pasture legume species to CMV and possible seed transmission of CMV in them were studied. Field experiments in 1994-1998 used spreader rows sown with CMV-infected lupin seed and natural aphid movement to spread the virus and so providing a high CMV inoculum pressure. Different genotypes of more than 30 different species from the genera *Biserulla, Cicer, Hedysarum, Lathyrus, Lens, Ornithopus, Pisum, Trifolium, Trigonella* and *Vicia* were grown. Plants with virus symptoms were counted at 2-week intervals in each test plot. Leaf samples were tested by ELISA to confirm visual diagnoses. To test for seed transmission seed was sown in trays or germinated in rolls of dampened absorbent paper. Seedlings or radicles were tested for CMV by ELISA.

Most of the >50 lentil genotypes evaluated were highly to moderately susceptible to CMV but some had low susceptibilities and few plants becaame infected (<10%) (eg. L-4, ILL5405). The >30 desi and kabuli chickpea genotypes evaluated varied in susceptibility to CMV, from very low (eg Amethyst mutant) to high (eg Sona, Dooen). In both lentil and chickpea, symptom severity varied between different genotypes. The grass pea *(Lathyrus ochrus)* and dwarf chickling *(L. cicera*) genotypes tested did not become infected and grass pea *(L. sativus*) was rarely infected. Field pea (*Pisum sativum*), narbon bean (*Vicia narbonensis*), faba bean (*V. faba*) and vetches (*Vicia* spp.)

sometimes became infected. Symptoms were usually mild in them except in bitter vetch (*V. ervillia).* With the pasture species, *Hedysarum coronarium* (cv. Sulla), *Trigonella balansae* and most *Trifolium* spp. had medium or high susceptibilities to CMV, but sea clover *(T. squarrosum*) and bladder clover (*T. spumosum)* had low susceptibilities. Many of the *Trifolium* spp. and *Trigonella balansae* had low to medium sensitivity and developed mild symptoms. *Biserulla pelecinus* had very low infection levels while pink and yellow serradella *(Ornithopus sativus* and *O. compressus*) were not infected. Seed transmission of CMV was confirmed in chickpea and lentil and found for the first time in five other clover species:- helmet (*T. clypeatum)* (0.05%), crimson (*T. incarnatum)* (4.8%), bladder (0.5%), and arrowleaf (*T. vesiculosum)* (1.2%) clovers, and narbon bean (0.8%). However, seed transmission was not found in seed samples of most of the other species.

QUANTIFYING THE RELATIONSHIP BETWEEN SEED SIZE IN NARROW-LEAFED LUPIN AND INCIDENCE OF CUCUMBER MOSAIC VIRUS

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To test the hypothesis that there is an inverse relationship between seed size and incidence of CMV seed infection in lupins, five commercial seed lots representing a range of CMV seed infection incidence levels were each sorted into 5 to 6 seed size classes by using different sieves (sizes 11, 13, 14, 15, and 16). Thousand seed weight (g) of each seed size fraction from each seed lot was determined by randomly counting out and weighing one thousand seeds. This procedure was repeated three times to obtain three replications for each seed size fraction. Using procedures regularly employed by the CMV testing service, each seed size fraction was tested five times (replications) to determine the presence of CMV seed infection (%) in each seed size fraction. Data were analyzed using the general linear model procedure and by linear regression (SAS Institute). The incidence of CMV seed infection (y) was regressed against thousand seed weight (x) for each seed size fraction.

The incidence of CMV seed infection was significantly higher ($P \leq 0.001$) in the small seed size fraction that passed through a size 13 screen and CMV seed infection was significantly lower as seed size increased for 4 of the 5 seed lots. In general, the incidence of CMV-infected seed decreased as seed size increased. There was a negative linear relationship between thousand seed weight and incidence of CMV-infected seed in 4 of the 5 seed lots. This indicates that the probability of detecting CMV in lupin seed can be improved by first sieving seed lot samples and then testing the smaller sized seed. Moreover, planting larger seed (seed remaining on a size of 15 screen or higher) would significantly reduce the risk of introducing CMV-infected lupin seed into growers' fields.

The above data supports the hypothesis that there is an inverse relationship between seed size and the percentage of seed-borne CMV. Therefore, sieving and testing only the smaller seed size fraction should improve the sensitivity of tests used to detect CMV in lupin seed lots. To test this second hypothesis, twenty seed lots that previously tested negative for the presence of CMV were selected arbitrarily and then re-tested for the presence of CMV by testing only the small seed size fraction that passed through a size 14 slotted sieve. Of the twenty seed lots previously testing negative for the presence of CMV, 10 of the 20 seed lots were found to test positive. Based on these

findings, it is recommended that the smaller seed size fraction (< size 14) from seed samples should be tested to increase the probability (sensitivity) of detecting CMV when it occurs in a seed lot.

SURVEYS OF VIRUSES AFFECTING PEPPER (*CAPSICUM ANNUUM L.*) IN TUNISIA

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In Tunisia, pepper is the third vegetable crop after potatoes and tomatoes. The main season of pepper production is from March to November in open field cultivation. The most used varieties are local populations saved by farmers or confirmed varieties produced by local or foreign seed companies. The pepper yields are generally low, and virus infections represent one of the most important constraints. Since 1994, the National Agronomic Research Institute (INRAT) has developed breeding programs to improve the pepper resistance to CMV, PVY and TMV of local cultivated types. Our aim is to evaluate the actual situation of pepper viruses in Tunisia to take it in account in breeding programs.

This paper presents the results of recent surveys in major growing pepper regions in Tunisia for viruses diseases naturally occurring.

Pepper samples were collected from July to October in open field. The major prospected production regions are as follows: North, North east, Coastal region (Centre) and from the Centre of Tunisia.

811 samples were collected in 98 from 52 sampled fields and were individually tested against five viruses CMV, PVY, AMV, TEV and PVMV. 100 samples from our laboratory collection were also tested. One hundred of the most representative samples presenting symptoms were sent to INRA Avignon and tested against TEV, PVMV, Poty- E, CVMV, CMV, and PVY.

The major disease is CMV, in all the prospected regions. The percentage of infection is higher in October than in early July corresponding to the end of season crop, ranged from 30 to nearly 100%.

For PVY, the percentage of infection varied from 0 to 52 %.

Some samples have shown multiple virus infection CMV and PVY or AMV. AMV was less prevalent, the percentage of infection ranged from 0 to 26%.

Only Two samples reacted slightly to Pepper Veinal Mottle Virus. The same for Tobacco Etch Virus, Three samples gave weak coloration.

Sample percentage showing virus like symptoms and who did not react in ELISA varied from 10 to 50%. These samples have to be tested against other antiserums, and indexed on indicator plants.

Samples tested at INRA Montfavet were in agreement with the results reported above for CMV, PVY, TEV and PVMV. These samples were also tested for CVMV and for Poty-E. Results demonstrated the absence of CVMV and low percentage of Poty E.

For suspicious samples (TEV, PVMV and Poty-E) biological indexing is necessary.

SESSION 6

MANAGEMENT AND CONTROL STRATEGIES

ORAL PRESENTATIONS

FIELD EXPRESSION, VARIABILITY, AND VIRUS STRAIN SPECIFICITY OF REPLICASE GENE-MEDIATED RESISTANCE TO POTATO LEAFROLL VIRUS

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Russet Burbank potato was transformed with seven constructs of the potato leafroll virus (PLRV) replicase gene. Two of the constructs also included the Cry IIIA gene from *Bacillus thuringiensis* ssp. *Tenebrionis*. Resistance of 512 transformant lines was evaluated in the field against the virus isolate source of the replicase gene. Resistance of selected lines was then tested against 66 isolates of the virus in the field selected from throughout the United States. The most virulent isolates were then used to evaluate resistance to plant-to-plant spread of the virus from infected weed hosts and potato as sources of virus. Resistance under natural exposure without insecticide treatments was also determined. Resistance was variable among transformed lines, but highly resistant lines were identified. Current season infection rarely developed in foliage of resistant lines but some tubers produced by inoculated, asymptomatic plants sometimes produced infected plants. Many virus isolates failed to infect the selected resistant lines, and the most resistant lines were infected by the fewest isolates. However, selected resistant lines were highly resistant even against the isolates capable of infecting them. Virus antigen content of systemically infected plants of the selected resistant lines was somewhat reduced compared with the non-transgenic Russet Burbank. Plant-to-plant spread from infected transgenic or non-transgenic potato plants to selected resistant plants was rare, but transmission to adjacent nontransgenic control plants occurred routinely. Two summer annual weed hosts of PLRV that are prevalent in potato fields of Northwest United States, Black Nightshade (*Solanum nigrum*) and Hairy Nightshade (*Solanum sarrachoides*), were much better sources of the major aphid vector of PLRV, *Myzus persicae*, than potato. PLRV antigen content in these weeds was lower than in potato. The capacity of these weeds to serve as virus sources for transmission to resistant plants is under study. Incidence of infection detectable by symptom development or clinical assays was rare among selected resistant lines in natural exposure plots where insecticides were not applied to control aphid vectors.

STRAIN STRUCTURE OF POTATO VIRUS Y (PVY) AND ITS IMPLICATIONS IN THE SPECIFICITIES OF PVY RESISTANCE IN PEPPER

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In any resistance-based strategy for plant virus control, a good durability of the resistance in the field or greenhouse is one of the most desirable traits. Numerous virus resistance genes are available in germplasm collections, and many of them have been used in breeding programs. When their durability performance in inbred lines is assessed, most the resistances sources provide short-to-medium range durable resistances, which are eventually overcome by new virulent virus pathotypes. A few resistance sources, however, have proven to be remarkably durable in cultivation, providing protection for several decades. Several explanations for this rather unpredictable resistance-overcoming phenomenon have been argued over the years, ranging from genetic instability of the resistance alleles in their new genetic background to a high mutation rate in the viral genome (specially in RNA viruses), thus constantly generating new viral pathotypes. Despite the partial explanations provided, a good model integrating all the variables playing a role in the system is, unfortunately, still lacking.

Several sources of resistance to potato virus Y (PVY) have been discovered in wild relatives of its cultivated hosts, which are mostly solanaceous vegetables, such as potato, pepper, tomato, tobacco, eggplant, etc.). The examples in potato and pepper are the best characterized so far. In a coordinated effort with other laboratories, we have focused on the study of PVY as a pepper pathogen, in order to understand how the different specificities of this system influence the different performances and durabilities found under cultivated conditions. Several parallel approaches have been adopted, including the study of the mechanisms underlying the action of several resistance genes, their genetic properties and gene-mapping, aphid-transmission efficiencies, and different levels of characterization of pepper-PVY isolates.

The genetic variability of the pathogen in a given viral population is an important component of the efficiency and durability of a given resistance source, inasmuch as it may determine the extent of the initial population that will be affected by the specificity conferred by the resistance considered. Through an estimation of the genetic distances between isolates - based on the restrictotypes of their coat protein genes as defined by five different restriction enzymes - we have studied the degree of genetic variability of typical pepper-isolates of PVY. The results obtained show clearly that the level of variability found is significantly smaller than that of potato or tomato isolates, to the extent that only one genetic strain has been found in pepper isolates. This suggests a host-driven restriction in the viral population. Regardless of this low variability, there is considerable pathotyping potential in this genetic strain, since three or four different pathotypes are found within it, as defined by the *pvr2* resistance allelic series.

The results obtained so far concerning the characterization of pepper-PVY isolates and pathotypes will be presented and discussed in the context of their contribution to our understanding of the performance of the resistance genes to PVY in pepper.

INMUNITY OF THE TRANSGENIC C-5 PLUM TO APHID-INOCULATION

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Over the last twelve years, much progress has been made by plant pathologists in controlling virus diseases and many reports have demonstrateded the efficiency of pathogen-derived resistance against plant viruses. Plum pox or sharka virus (PPV) is causing considerable yield losses for stone fruit production in Europe. First described in Bulgaria, dissemination of infected plant material and by aphid vectors have led to the spread of PPV throughout Europe. Search for natural gene resistance by conventional breeding revealed a few examples of apricot (Dosba *et al.*, 1994, OEPP/EPPO Bulletin, 24, 691-696), plum (Kegler and Hartmann, 1998, in Hadidi *et al.*: *Plant virus diesase control*, APS Press, Minnesota, 616-628) that have been clearly identified as tolerant to PPV infection. In situations such as these, fruit production can be exploited but the trees remain infected and potential sources of PPV. For this reason, the concept of non-conventional protection based on the integration of capsid protein gene into plants offers the opportunity to develop this strategy against PPV. Transgenic plums have been shown recently under confined greenhouse conditions to be resistant to PPV (Ravelonandro *et al.*, 1997, *Plant Disease*, 81, 1231-1235). Transgenic C-5 plum has been propagated vegetatively and resulting clones have been inoculated by PPV with the aphid vector *Myzus persicae* Sulz. After inoculation, plants were investigated for virus replication at an early stage. Regardless of the different sizing of scions tested, IC/PCR and western-blotting experiments showed that the majority of control plants (transgenic or non-transgenic) known for their susceptibility to PPV were positive. However not one plant of transgenic clone C-5 has been found to be sensitive.

The significance of such reaction is corroborated by the results under field studies in Skierniewice (Poland). Under high pressure of inocula in an experimental orchard with indigenous vectors like *Hyalopterus pruni*, *Brachycaudus cardui*, *B. helichrysi* and *Myzus persicae* we observed that the majority of plums known for their susceptibility to PPV infection showed symptoms and conversely not one C-5 plum has been infected during three years. Although the observations of these plants are still continuing, these experiments provide clear evidence for the reaction of the transgenic C-5 plum that corroborates the previous results in confined greenhouse conditions. The co-suppression mechanisms proposed for inducing the immunity of these transgenic clones are active in fully expanded leaves and did not permit the virus to replicate and to spread systemically in the whole plant. Referring to the biochemical characteristics of the transgenic plum C-5 the involvement of the viral transgene products (DNA or RNA) in foliar tissue can be regarded as determinants in the development of this effective resistance. These findings are interesting because they support the efficiency of the pathogen-derived approach against PPV.

CHARACTERIZATION OF ATTENUATED ENGINEERED VIRAL cDNA OF ZUCCHINI YELLOW MOSAIC VIRUS FOR CROSS PROTECTION IN CUCURBITS

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Zucchini yellow mosaic virus (ZYMV) is a member of the *Potyvirus* genus and causes devastating epidemics in commercial cucurbits world-wide. Development of virus-resistant cultivars by classical breeding or by the introduction of viral nucleic acid sequences into the plant genome are difficult and slow. The alternative method of cross protection is currently being used successfully with the natural mild strain ZYMV-WK in Israel. However, maintenance and growth of the source inoculum and the development of highly infectious cucurbit seedlings in the greenhouse was difficult using the ZYMV-WK mild strain for commercial application.

A full length infectious clone of an attenuated ZYMV-AG1 isolate were constructed, and tested successfully by particle bombardment. Infection of cucurbits with the engineered ZYMV-AG1 strain changed the symptoms dramatically from severe to mild in squash, and to symptomless in cucumber, melon and watermelon. The engineered virus was found to be very stable and so far no revertant virus has been found during several passages and long periods of incubation. The AG1 strain was detected 5-7 days post-inoculation and accumulated in cucurbits to similar levels to those found with the wild type JV strain. The attenuated ZYMV-AG strain harbors an additional point mutation that abolishes transmission of the virus between plants by aphids. This characteristic reduces the possibility of potential ecological problems. Infection with the AG1 strain was found to protect cucurbits against the infection by the severe strain in cross protection assays. Field experiments in squash and watermelon demonstrate a protective effect of the AG1 strain. No differences in weight or number of fruit were found between plots treated with naturally weak (WK) and engineered cloned virus (AG1), and the control plots.

RESISTANCE TO THE CAUSAL AGENT OF BLACKCURRANT REVERSION DISEASE AND TO ITS GALL MITE VECTOR PROVIDES EFFECTIVE CONTROL OF THESE ORGANISMS IN THE FIELD, BUT FOR HOW LONG?

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An assessment was made over 5 years of the response of 10 blackcurrant genotypes, differing in resistance to the causal agent of blackcurrant reversion disease (BRD) and to its gall mite vector, in field trials in Scotland and Finland containing high inoculum levels of the two organisms. In Scotland, the infector plants contained large numbers of gall mites (*Cecidophyopsis ribis*) and were

infected with the European form of BRD; in Finland, infector plants contained a different species of gall mite (*C. spicata*) and the severe Russian form of BRD. At both sites, almost all plants of cvs Ben Alder, Ben Lomond, Ben Tirran, ÷jebyn and an SCRI selection F4/1/66, which are susceptible to gall mite and BRD, were affected by each of these organisms. The cv. Foxendown, which contains gene Ce conferring apparent immunity to *C. ribis*, was free from galls and failed to develop distinctive BRD symptoms at both sites. The cvs Rus and Neosypajuscajaija, which contain gene P, reported to confer resistance to *C. ribis*, were affected more slowly by mites than the mite-susceptible genotypes and showed a smaller number of galls per plant. Also, they were infected with BRD more slowly than some mite-susceptible genotypes although by the end of the experiment, most plants were affected by BRD. All plants of cvs Golubka and Ben Gairn, which are resistant to the agent of BRD, remained free from distinct BRD symptoms at both sites despite the fact that plants contained galls. These data indicate the superiority of gene Ce over gene P for resistance to gall mites with the added benefit that the virtual immunity to gall mites provided by gene Ce also provides a high level of protection against infection with BRD. Very recently, several new species of *Cecidophyopsis* mites have been found on different species of Ribes and identified by genetic fingerprinting. Some of these mite species are known to cross-colonise different *Ribes* species but the host range of others is as yet undetermined; their ability to vector the agent of BRD is also unknown. The relative merits of the different forms of plant resistance to gall mites and to BRD in blackcurrant are discussed in relation to present control methods for these two organisms and in the light of these recent findings of previously uncharacterised eriophyid mites on *Ribes* species.

OPTICAL BARRIERS: AN INNOVATIVE IPM TOOL FOR THE CONTROL OF INSECT PESTS AND VIRUS DISEASES IN PROTECTED CROPS

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Vision behaviour of insects is linked to the chain of events which begins with their orientation to the plant from a distance and ends with their establishment on plants for feeding and oviposition. By interfering with different links along this pathway contact between the vector and the plant which lead to virus infection is prevented. Photo-selective greenhouse cladding materials can serve as filters to eliminate parts of the light spectrum thus affecting insects behaviour. In experiments carried out during the last three years tomato grown in small (6x6x2.7m) 'walk in' tunnels covered with UV-absorbing polyethylene sheet were highly protected against infestation with the silver leaf whitefly *Bemisia argentifolii* Bellows and Perring and infection with tomato yellow leaf curl geminivirus (TYLCV) vectored by this insect. The numbers of whiteflies trapped under the conventional sheets were 4-10 times greater than the numbers that were recorded under the UV-absorbing films. UV-absorbing polyethylene films reduced TYLCV disease incidence between 20% to 30%, respectively, while disease incidence in control tunnels covered with ordinary IR films reached 60%. The results in unsprayed tomato grown in commercial-sized tunnels covered with UV-absorbing sheets were dramatic: 1% infection with TYLCV under the UV-absorbing sheets as compared to 87% in the control tunnels covered with the ordinary films. In trials carried out on green herbs grown commercially in 'walk in' tunnels the use of UV-absorbing films considerably reduced the incidence of three major insect pests: *B. argentifolii*, *Frankliniella occidentalis* Pergande and *Liriomyza trifolii* Burgess. This reduction in pest populations in return reduced the need for chemical pesticides. Plastic screens with UV-absorbancy in the UV-A and UV-B range ('bionets'), were compared with conventional nets of the same mesh size for their protective capacity against vegetable insect pests and spread of virus diseases. Conventional and 'bionet' screens with densities

of 16- and 30- mesh were not effective in preventing the penetration of *B.argentifolii* into 'walk-in' tunnels covered with these nets. However, 'bionet' screens of 50- mesh size drastically reduced the penetration of whiteflies into tunnels as well as the spread of TYLCV. Fifty days after planting 30% disease incidence was recorded in unsprayed tomatoes grown under 50-mesh 'bionet' screens, compared with 80% incidence in tunnels covered with conventional 50-mesh net. The mechanism behind the above mentioned protection phenomena is not clear yet, however it is suggested that elimination of UV from the light spectrum is interfering with the orientation capability of whiteflies and other insect pests and is controlled by photoreceptors located in the insect's eye. Our studies indicate that the use of UV-absorbing polyethylene films and nets can act as efficient optical barriers against the spread of insect pests and virus diseases. This new strategy may serve as an element of IPM and may contribute to a reduction in the use of chemical control.

DEVELOPMENT OF INTEGRATED DISEASE MANAGEMENT STRATEGIES FOR TWO NON-PERSISTENTLY APHID-TRANSMITTED VIRUSES INFECTING LUPIN CROPS

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Integrated virus disease management strategies combine the various control measures available. The different measures combine to minimise the source of virus infection, maximise the suppression of virus spread within the crop and minimise any impact on yield. The effects of using different potential control measures on virus spread and grain yield were studied over a 10-year period in field experiments at a range of sites in different climatic zones of Western Australia. This work involved two non-persistently aphid-transmitted viruses, cucumber mosaic (CMV) and bean yellow mosaic (BYMV), that cause serious diseases of narrow-leafed lupin (*Lupinus angustifolius*), the most important grain legume crop in Australia. CMV is introduced into lupin crops by sowing virus-infected lupin seed while BYMV spreads into the growing crop from adjacent clover pastures.

Through sowing seed lots with different levels of virus infection in field experiments, we have established for lupins the threshold levels of CMV seed infection that are acceptable in different risk zones. For high risk zones the threshold is <0.1% but for low risk zones it is <0.5%. Growers in high risk areas can obtain their seed from areas of low risk. A non-host perimeter buffer decreased BYMV spread into lupin crops from external virus sources but was of no benefit with CMV as the virus source is internal.

With both viruses, early plant canopy cover decreased virus spread by shading out virus-infected source plants within the crop, denying aphids access to these source plants and decreasing aphid landing rates. Especially with BYMV, it also benefited the crop through compensatory growth of healthy plants filling in space left by poorly growing virus diseased plants. Early plant canopy cover was promoted by early sowing, high seeding rates, narrow row spacing and banding fertiliser below the seed sown. With both viruses, the extent of spread decreased with increasing seeding rate and plant density. Narrow row spacing decreased spread of BYMV due to delayed canopy closure causing decreased aphid landing rates but increased spread of CMV due to greater shading out of seed-infected source plants within the denser wide rows. Before canopy closure, stubble

groundcover was beneficial by decreasing aphid landings. With both viruses, the greater the amount of stubble the greater the decrease in virus spread. Application of a range of different types of insecticides was of limited benefit in decreasing spread of either virus.

The integrated disease management strategies developed for CMV and BYMV in lupins include sowing seed with minimal virus content for (CMV), perimeter non-host buffers (for BYMV), early sowing, increased seeding rate/plant density, modified row spacing, direct drilling into retained stubble, banded fertiliser application, improved weed control and isolation (both viruses). They have been widely adopted by the lupin industry in Australia.

IPM AND PLANT VIRUSES: TRYING TO FIT A SQUARE PEG INTO A ROUND HOLE?

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Traditional IPM tactics are often implemented when critical thresholds have been reached. Scouting for pests and assessment of damage and damage potential with an eye towards an economic injury level are the cornerstones of many current IPM programs in agro-ecosystems. Unfortunately, insect-vectored plant viruses, particularly non-persistently transmitted viruses, have never been amenable to such a "wait and see" attitude because thresholds can be extremely low and remedial tactics are ineffective. With as few as 1% of plants infected through seed and high vector activity rates, 100% of the crop can become infected (Ruesink & Irwin 1986) during the season, with varying rates of yield loss depending on the timing of infections.

Timing is crucial here as in other IPM programs, but more so because once a plant becomes infected with a virus, there is no way to mitigate the damage and it becomes available as a source for further spread of the virus. In addition, the earlier the plant becomes infected, the greater the effect on yield and seed quality. Plants infected before flowering have a greater chance of producing seed-borne progeny if they produce seeds at all.

Vectors are perhaps even more important than the plant and disease in such systems, because aside from host plant resistance to the virus, little can be done to affect the spread of an epidemic without affecting the vector. For most non-persistently transmitted viruses, there are numerous transient vector species, each with its own bioecology to consider and vector propensity to transmit the virus (Irwin & Ruesink, 1986). Managing plant viral epidemics then involves juggling the management of the likely vectors for an area. However, the mix of these vectors can change from season to season for no apparent reason.

One of the goals of IPM is to reduce pesticide inputs, even though part of the traditional arsenal available to IPM practitioners is the judicious use of chemical control of pests. However, because most chemicals cannot act quickly enough to mitigate the actions of vectors of non-persistently transmitted diseases and in fact often cause additional movement, their use is undesirable and the mangement approach needs to be preventative rather than remedial.

Preventative IPM for insect-vectored plant viruses is thus based on knowledge of the vectors, their bioecology and flight patterns, propensity to transmit the virus in question, and a variety of

cultural controls that may delay the onset of epidemics to a point where infections come mainly after flowering when the plant is less susceptible to yield loss.

POSTER PRESENTATIONS

CAPACITY OF AN ALL-ROUND PROTECTING AGENT IN AN INTEGRATED PLANT MANAGEMENT STRATEGY

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The integration of several control strategies for managing plant-parasitic nematodes and foliar feeding pests is a necessary approach to minimise the risk of economic loss and to improve the financial revenue of individual farmers. The use of beneficials against insect pests is an effective tool to decrease damage to greenhouse crops. Biological control alone, however, is not sufficient and the delayed control increases the risk of indirect damage due to virus transmission.

The possibility of a multiple delivery of Vydate (reg.) (a. i. Oxamyl), via a drip-irrigation system provides an effective strategy to restrict the distribution of the control agent. The highly water-soluble Oxamyl easily moves down to the target soil regions of highest root and nematode abundance. Oxamyl treatment to the soil for protection against nematodes (*Meloidogyne incognita*) provides at the same time foliar protection against insects. This highly systemic control agent moves along the xylem within an extremely short time (10 to 12 hours) to the foliage where it controls the important virus vectors *Bemisia tabaci***,** *Myzus persicae* and thrips (*Thrips tabaci*). Even other pests, including leafminers (*Liriomyza trifolii*) and mites (*Tetranychus urticae*) are controlled effectively depending on the application frequency. The systemic capability of Vydate (reg.) can be seen in a wide variety of different plant species.

By comparing a conventional broadcast foliar spray to a Vydate (reg.) application via drip irrigation, beneficials remain unaffected despite chemical protection. Thus drip application with Vydate (reg.) provides:

- Good crop protection whilst using significantly reduced rate per hectare to control nematodes and insects
- No effect on beneficial insects
- A cost effective and environmentally safe system for IPM

SELECTION OF RESISTANCE-BREAKING STRAINS OF TOMATO SPOTTED WILT TOSPOVIRUS

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In glasshouse tests, sap from plants infected with 15 different isolates of tomato spotted wilt tospovirus (TSWV) from three Australian states was inoculated to nine genotypes of tomato carrying TSWV resistance gene *Sw-5* or one of its alleles. A further two resistant tomato genotypes were inoculated each with four isolates. The normal response in resistant genotypes was development of necrotic local lesions in inoculated leaves without systemic invasion, but 22/752 plants also developed systemic reactions in addition to local hypersensitive ones. Using cultures from two of these systemically infected plants and following four cycles of subculture in TSWV-resistant tomato plants, two isolates were obtained that gave susceptible type systemic reactions but no necrotic spots in inoculated leaves of resistant tomatoes. When these two isolates, Da_{WA}-1d and To_{TAS}-1d, were maintained by repeated subculture for 10 successive cycles in *Nicotiana glutinosa* or a susceptible tomato genotype, they still induced susceptible-type systemic reactions when inoculated to resistant tomato plants. They were therefore stable resistance-breaking isolates overcoming gene *Sw-5*. When resistance-breaking isolate Da_{WA}-1d multiplied together with original isolate Da_{WA}-1 in susceptible tomato, it was fully competitive with the original isolate. However, when Da_{WA}-1d and To_{TAS}-1d were inoculated to TSWV-resistant *Lycopersicon peruvianum* lines PI 128660R and PI 128660S and to TSWV-resistant *Capsicum chinense* lines PI 152225, P1 159236 and AVRDC C00943, they failed to overcome the resistance, producing only necrotic local lesions without systemic infection. Thus, although the ease of selection, stability and competitive ability of resistance-breaking isolates of TSWV is cause for concern, *L. peruvianum* lines and *C. chinense* lines are available which are effective against them.

The effectiveness of the resistance to TSWV in nine tomato genotypes was examined in a field experiment. Spread was substantial in the susceptible control genotype infecting 42% of plants. Resistance was ineffective in cv. Bronze Rebel, 26% of plants developing infection. In contrast, it held up well in the other eight resistant genotypes with only 1-3 or no plants of each becoming infected. Accumulated numbers of *Thrips tabaci*, *Frankliniella occidentalis* and *F. schultzei* were closely correlated with TSWV spread.

CHARACTERIZATION OF SOYBEAN MOSAIC VIRUS COAT PROTEIN-MEDIATED RESISTANCE IN TRANSGENIC SOYBEANS

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Soybean mosaic virus (SMV) is present in all major soybean production areas of the US. This disease can greatly reduce yield, seed quality, and oil content. Although seed size and weight of soybeans can be greatly reduced by early infection, late infection has little effect on these yield components. Thus, breeding for resistant cultivars that can reduce or delay the time of infection

should provide a significant yield benefit and enhance net return. Although there are some resistant cultivars now available for use in the southern US, no single cultivar is resistant to all SMV strains.

Pathogen-derived resistance, through the use of coat protein (CP)-mediated resistance, is one approach that could be used to produce soybean plants resistant to SMV. In general, transgenic plants that express the CP gene have reduced symptom severity and delayed symptom expression. This suggests that this form of resistance may have the potential to reduce the rate of plant-to-plant virus spread in soybean fields, thereby delaying the time of infection by SMV.

In cooperation with Pioneer Hi-Bred International Inc., we developed soybean plants transformed with the CP gene of SMV strain N, and obtained four transgenic soybean lines. PCR and southern blot analyses were used to detect the transgene. A single CP gene transcript was detected in all four transgenic lines and western blots showed CP expression in three lines. Northern analyses demonstrated transcription in all lines. Selfing of plants yielded homozygous transgenic plants.

To assess resistance of soybean plants to SMV, soybean plants were mechanically inoculated with purified SMV strain N at soybean growth stage V2. Inoculation was performed by placing 10 ul of inoculum on one newly expanded leaflet that had been previously dusted with Carborundum (600 mesh). Plants were observed for 40 days; time of symptom appearance and symptom severity were recorded. Plants with appearance of light and dark areas on the newly expanded leaves were judged as symptom positive. A biotin avidin ELISA assay was conducted to confirm visual results.

Based on infection efficiency, all four transgenic lines exhibited degrees of resistance to SMV infection. The incubation period in transgenic line 6-13 was 21 days, which was 9 days longer than in the non-transgenic control. Transgenic lines 7b-11 and 3-24 failed to develop SMV symptoms. Mosaic symptoms expressed in all sympotmic transgenic plants were less severe than those observed in the non-transgenic control. In all transgenic and non-transgenic plants, symptom expression was consistent with ELISA results. Transgenic line 7b-11 has the same number of CP gene insertion events as transgenic line 3-24, which accumulated the highest apparent level of transgene transcripts and coat protein. Unlike line 3-24, however, line 7b-11 accumulated the lowest apparent transcript level and there was no detectable CP. Nevertheless, both transgenic lines 7b-11 and 3-24 expressed a high degree of resistance to SMV. We suggest that resistance in line 7b-11 may be conferred at the RNA level.

Experiments to quantify the effect of transgenic lines on acquisition and transmission efficiency by the green peach aphid are currently underway. Field experiments to quantify the effect of transgenic soybean populations on the temporal and spatial spread of SMV will begin in June 1999 using methods previously published (Phytopathology 78: 895-901).

STUDIES ON THE TRANSLOCATION OF TOMATO SPOTTED WILT TOSPOVIRUS IN POTATOES

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Tomato spotted wilt virus (TSWV) causes sporadic but important epidemics in Australian seed potatoes. Whilst it is known that TSWV transmission from infected potato plants to tubers can be inefficient, certification standards rely on visual and ELISA testing of plants during the growing season. If TSWV incidence in tubers could be reliably predicted from field inspection then certification standards which reflect the actual risk of this virus could be set. TSWV tuber transmission in Russet Burbank (RBK) and Shepody (SHP) was evaluated. An erratic distribution of TSWV was found in infected tubers through ELISA testing of tissues. TSWV was found most frequently in the tuber centre and rose end, whereas the heel end and tissues around the vascular tissue and eyes frequently tested virus-free. Tuber symptom expression varied with cultivar. SHP tubers often showed necrotic surface lesions and severe internal necrosis. Symptomatic tubers always contained TSWV but a portion of infected tubers was symptomless. In RBK, necrosis was infrequent and always mild. Many tubers from infected plants showed growth irregularities which were not associated with tuber infection, and reflected poor plant growth. TSWV infection also varied markedly with cultivar and RBK plants produced 36% infected tubers and SHP 86% infection. Tuber infection was further influenced by the extent of infection in the parent plant. The proportion of infected tubers was reduced where at least one stem of the parent plant was not infected (29% compared to 41% in RBK plants with all stems infected and 84% compared to 91% in SHP). TSWV-infected tubers were then grown-on to determine the transmission rate from infected tuber to plants. Again a marked difference was found between cultivars and only 20% of RBK and 80% of SHP tubers gave rise to at least one infected shoot. These infections were often delayed by up to 4 weeks with shoots initially growing as virus free stems. Under field conditions such tuber-borne infections could be confused as primary infections. Current tests are assessing the effect of physiological age of the plant at infection and any virus strain differences on tuber transmission efficiency.

EVALUATION OF REFLECTIVE MULCHING IN COMBINATION WITH INSECTICIDE SPRAYS FOR CONTROL OF APHID-BORNE VIRUSES OF FLOWER BULBS

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Reflective mulching was examined as an augmentation or replacement for chemical virus vector control in flower bulb production. Replicated plots of bulbous iris, cv. Professor Blaau, with 12.0% infection of iris mild mosaic virus (IMMV) and tulip, cv. Gander, with 4.4% infection of tulip breaking virus (TBV) were planted at commercial densities. Treatments were untreated control, mulching (plots surrounded by 90cm of silvered plastic), weekly insecticide sprays (fenvalerate at 330 ml a.i./ha) and a combination treatment in tulips, and these plus weekly mineral oil sprays (Caltex Lovis, 99% summer oil, at 20 L/ha) applied alone, in combination with mulching, and at half

rate with insecticide in iris. Aphid activity was assessed above mulched and non-mulched treatments with yellow sticky traps. Flower stem lengths were measured at flowering. At harvest the largest bulb from each plant was weighed and stored before replanting in an insect-proof glasshouse. Leaf material was then tested for TBV and IMMV by ELISA. Fewer aphids were trapped above mulched than non-mulched plots demonstrating a deterrent effect. This decrease was greater for *Myzus persicae* (85%) than *Macrosiphon euphorbiae* (42%) or non-vector species (48%). Control iris plots (98% infection), had significantly more IMMV than other treatments. The mulching and insecticide combination had the least IMMV (35%) with other treatments intermediate in activity (mineral oil, 42%; insecticide, 52%; oil with mulching, 55%; half-rate oil with insecticide, 66%; and mulching, 69%). With little spread of TBV (3% infection across plots) there was no significant effect of treatments on TBV spread, although means suggested a similar trend to that for IMMV. Mulch treatments had no effect on stem length or bulb weight of either crop, but the insecticide treatment (tulips) and all oil treatments (iris) significantly reduced both stem length and bulb weight. Tulips and iris are high value and high virus risk crops which have an erect morphology. They do not sprawl over the mulch during growth and so do not reduce its efficacy. This makes them ideal subjects for this management strategy. This work suggests that enhanced virus management in flower bulb production may be achieved by incorporating reflective mulches within current virus control strategies.

CAN PYRETHROID AND IMIDACLOPRID INSECTICIDES BE USED TO CONTROL SPREAD OF CUCUMBER MOSAIC VIRUS IN LUPIN?

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Five field experiments in 1995-1997 investigated the effects of alpha-cypermethrin and imidacloprid insecticides on spread of cucumber mosaic virus (CMV) in narrow-leafed lupin (*Lupinus angustifolius*). Single or double foliar sprays of both chemicals were used and imidacloprid was also applied as a seed dressing. CMV was introduced to plots by sowing 7% infected lupin seed and control plots were sown with healthy seed. Non-host buffers 25-30 metres wide separated the plots. Seed-infected plants were the primary infection source for spread by aphids. CMV incidence was assessed by counting plants with characteristic symptoms and testing randomly collected leaf samples by ELISA. Aphids were counted *in situ* on growing tips.

Myzus persicae was the most abundant colonising aphid species, but *Aphis craccivora* and *Acyrthosiphon kondoi* also colonised the lupins. Aphids were never abundant enough to cause direct feeding damage. More aphids colonised CMV-infected plants than healthy ones. Alpha-cypermethrin was more effective at suppressing colonisation by *A. kondoi* or *A. craccivora* than by *M. persicae*. Imidacloprid was more effective at diminishing numbers of *M. persicae* than of *A. kondoi* or *A. craccivora*. Over 85% of *M. persicae* collected from sprayed plots were R_1 (resistant) or R_2 (highly resistant) type aphids and therefore resistant to pyrethroids and organophosphates.

CMV symptom assessments underestimated CMV infection incidence as determined by ELISA by more than threefold. Single or double applications of 25g a.i./ha alpha-cypermethrin significantly ($P<0.05$) decreased colonising aphid numbers and the rate of CMV spread.

Applications of imidacloprid (seed dressings or foliar sprays), methamidophos or triazamate affected aphids in a similar way to alpha-cypermethrin, but did not significantly decrease CMV incidence. Virus spread between plots diminished measurable chemical treatment effects. Plots sown with healthy seed always out-yielded those sown with infected seed. Significant grain yield increases due to applied chemicals were recorded only in one experiment, where alpha-cypermethrin at 25g a.i./ha increased yields by 60%. However, in all experiments the highest yields were recorded when this chemical was sprayed. A clear linear relationship (negative) was found between % CMV infection and grain yield. With one exception, there were no significant chemical treatment effects on seed weight or CMV transmission into harvested seed in plots sown with infected seed.

Considering that insecticide resistance to pyrethroids was present in *M. persicae*, these results suggest that sowing healthy seed and using recommended cultural management practices that minimise spread should remain as the mainstays of successful CMV management in lupins. A foliar application of 25g a.i./ha alpha-cypermethrin can be used to complement this integrated disease management strategy by further diminishing CMV spread, but should not be relied upon alone. Based on these experiments use of imidacloprid could not be recommended for CMV control.

STUDIES ON THE RESISTANCE OF A *CUCUMI MELO* ACCESSION TO WATERMELON MOSAIC VIRUS-2

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Melon (*Cucumis melo* L.) is an important vegetable crop in Spain, ranking first by area and second by economic value. Spain is the fifth largest melon producer in the world by area (47,000 ha) and by production (845,000 t) (F.A.O., 1996). Most (75%) of Spanish melon production is done in the open in the eastern and southern halves of the Iberian Peninsula, whereas 25% is produced in plastic-houses in a small region of the southeastern coast.

Mosaic diseases caused by aphid-borne viruses are an important problem of melon production in Spain, and watermelon mosaic virus-2 (WMV-2) is one of the most widespread viruses in field-grown melons (Luis-Arteaga *et al.*, 1998). WMV-2 is naturally transmitted in a non-persistent manner and can cause appreciable losses in production if plants are infected in early growth stages. Plant protection strategies based on chemical treatments for vector control or crop management are not effective to limit disease losses. Host resistance offers the best mean for control. The availability of resistance against WMV-2 in melon is very limited and no commercial cultivar resistant to this virus has been obtained. Therefore, a project was undertaken to search for sources of resistance/tolerance against WMV-2 in *C. melo* and wild relatives in the germplasm collection of "La Mayora"(CSIC). As a result of a first screening, one accession of *C. melo* from Zimbabwe, TGR, was selected for further studies on resistance. Plants of TGR were tested against three isolates of WMV-2, M-116, M486, and M507, collected in Spain from mosaic epidemics that occurred on melon in Valencia, Murcia, and Ciudad Real, respectively. Thirty plants per combination were inoculated mechanically in a greenhouse (*c*. 25ºC day, 20ºC night, 16 h photoperiod) using the cultivar 'Bola de Oro' as susceptible control. TGR showed partial resistance to WMV-2 infection: only a few plants developed symptoms of virus infection in TGR with any of these isolates and symptoms were very mild compared to the strong green mosaic and 'bubbling' observed in 'Bola de Oro'. M116 seemed to be more aggressive than M486 or M507 in TGR and, therefore, was selected for further studies on the genetics of resistance. Vector transmission experiments were also

performed: ten plants of each TGR and the susceptible control 'PMR 45' were inoculated with M-116 using *Aphis gossypii* as transmitter; none of the TGR plants was infected whereas 60% of the'PMR45' were infected. The genetic basis of resistance in TGR was studied by mechanical inoculation of M116 to both TGR and 'Bola de Oro' parents, as well as F_1, F_2 and backrosses to TGR and 'Bola de Oro'. Results will be presented that suggest the possible implication of two dominant genes in the resistance of TGR to WMV-2.

EVALUATION OF FIPRONIL FOR CONTROLLING TOMATO SPOTTED WILT TOSPOVIRUS TRANSMISSION

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There are several reports indicating that the the insecticide fipronil can control *Frankliniella occidentalis* very effectively**,** although it is not known if the spread of tomato spotted wilt virus (TSWV) can be reduced by using this product. Therefore, we evaluated the efficacy of fipronil for reducing the transmission rate of TSWV by its main vector, *F. occidentalis.*

First, a Petunia leaf disk assay was used to test the efficacy of the product. The insecticide treatment was applied by immersing a 1-cm diameter leaf disc for 15 minutes into a solution with a concentration of 5 g/Hl of active ingredient (fipronil, and formetanate, used as standard). Viruliferous thrips obtained after access to TSWV-infected pepper leaves were placed on the Petunia leaves for a 24h inoculation access period. The results indicated that in 2 of 3 assays fipronil reduced but did not suppress transmission.

In a second series of experiments the insecticide solutions were applied at a dose of 5 g/Hl of active ingredients on the leaves of pepper (cv. "Yolo Wonder" and "Negral") and tomato (cv. "Tres Cantos") seedlings. Then, 10 viruliferous adults were released on each seedling. After 30 days, the number of TSWV-infected plants was assessed by ELISA. Viruliferous thrips were obtained by two different methods: a. larvae exposure during 24h to a TSWV-infected source and subsequent development on green bean pods until adult emergence; b. larvae completing their whole life cycle on *Datura stramonium* plants infected with TSWV. In the case of pepper (cv. "Yolo Wonder"), fipronil reduced the incidence of TSWV when compared to the untreated control (χ^2 = 4.8, P = 0.028), but there were no statistical differences between the plants treated with formetanate and the untreated control. In the case of tomato (cv. "Tres Cantos**"**), we found similar results as for 'Yolo Wonder' pepper (χ^2 = 4.5, P = 0.00339). However, no statistical differences between treatments were obtained with pepper (cv. "Negral"), probably because this cultivar is very sensitive to TSWV. In this case, very high transmission rates were obtained for both insecticide-treated and untreated plants.

Therefore, fipronil may reduce the transmission rate of TSWV by *F. occidentalis*, although this was not the case when the cultivar appeared to be very susceptible to the virus. Experiments under field conditions are required to clarify the usefulness of fipronil for the control of TSWV.

BARRIER CROPS TO CONTROL NON-PERSISTENTLY TRANSMITTED VIRUSES OF PEPPER CROPS IN SPAIN

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This work reports on the efficacy of barrier crops as a preventive control measure against potato virus Y (PVY) and cucumber mosaic virus (CMV) infecting pepper crops, and their effect on pepper yield. A comparison of aphid landing rate betwen plots surrounded by different crops was also made.

Laboratory assays with two main aphid vector species (*Myzus persicae* and *Aphis gossypii*) were conducted to find out if sorghum or maize plants could act as natural sinks of CMV and PVY. We found out that aphids subjected to an acquisition access time of 5 min on infected sources significantly reduced their transmission rate to pepper after probing on sorghum or maize plants.

Field experiments were conducted during 1997 and 1998 in Arganda del Rey, Madrid. using a latin square design with the following treatments: pepper plots surrounded by 4 rows of sorghum, pepper plots surrounded by 4 rows of vetch, pepper plots sprayed with a paraffin oil (Vektaphid) and surrounded by 4 rows of sorghum, and pepper plots without barriers (control). To increase virus inoculum pressure PVY- and CMV-infected plants were placed uniformly around all the plots. The incidence of PVY was determined by DAS-ELISA and CMV by visual observation. Aphid landing activity was monitored using green tile traps that were collected weekly from May to September.

Results in 1997 indicated that barrier crops were unable to significantly ($P>0,05$) reduce virus infection rate, although PVY infection was delayed in the plots sprayed with paraffin oil (Vektaphid) and surrounded by 4 rows of sorghum. Yield obtained (total and commercial fruit weight) was significantly ($P< 0,05$) lower in the pepper control plots than in the pepper plots protected by sorghum, vetch or sorghum + mineral oil. This increase in yield observed in pepper plots surrounded by barriers crops could not be related to a reduction in the total number of plants infected with PVY or CMV. Some other factors might have been responsable for this yield increase (reduced evapotranspiration, temperature, etc.). Aphid traps catches showed that the landing rate was similar in the protected and unprotected pepper plots, indicating that barrier crops may act as a natural sink of virus infection but are unable to reduce the total number of aphids landing on the protected crop.

EVALUATION OF ELITE BREEDING LINES HAVING RESISTANCE TO RICE TUNGRO VIRUSES

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Twelve elite breeding lines were evaluated for resistance to rice tungro viruses in replicated 8m x 8m plots at two sites in the Philippines, India and Indonesia from 1995 to 1998. The breeding lines were developed at the International Rice Research Institute in the Philippines using five unimproved rice varieties and two wild rices as virus-resistant donors. Breeding lines with resistance derived from Utri Merah (International Rice Germplasm Center accession no. 16680) had low infection with rice tungro bacilliform and rice tungro spherical viruses at each of the six trial sites, suggesting that resistance is likely to be effective at a wide range of locations in South and Southeast Asia. Two breeding lines derived from ARC11554 (IRGC accession no. 21473) showed good resistance to rice tungro viruses in Philippines and Indonesia but not in India. The results are discussed in relation to the need to deploy rice varieties with durable resistance to rice tungro disease.

EVALUATION OF TRANSGENIC WINTER WHEAT FOR RESISTANCE TO BARLEY YELLOW DWARF AND WHEAT STREAK MOSAIC VIRUSES

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Immature embryo-derived callus tissue of two soft white winter wheat cultivars (Daws and Lambert) was subjected to transformation via microprojectile bombardment. Genes utilized for transformation include barley yellow dwarf virus (PAV serotype) coat protein (*BYDV-CP*), wheat streak mosaic virus coat protein (*WSMV-CP*), and a gene derived from the yeast *Schizosaccharomyces pombe* (*pac1*) that encodes an enzyme with specific activity as a double-stranded ribonuclease. It is expected that if the *pac1* gene is expressed in transgenic plants, the enzyme will degrade dsRNA replicative intermediates of ssRNA viruses such as BYDV and WSMV. The *bar* gene for phosphinothricin resistance was used as a selectable marker. PCR assays were used to detect introduced genes in the transformed plants. Stable, independent integration of the genes was detected in subsequent generations using PCR. Expression of *WSMV-CP* was confirmed using Southern, northern and western blot analyses.

Virus challenge tests have been conducted in the greenhouse and field. Plants that showed phenotypic resistance following challenge with WSMV were identified among the WSMV- CP transformants. Some lines showed stable resistance in subsequent generations, while others did not. Field tests with transgenic WSMV-CP lines suggest that following virus challenge some lines have yields comparable to non-inoculated control plants. The BYDV-CP transformants showed a range in expression from susceptible to partially resistant after being challenged with BYDV.

Several transgenic *pac1* lines derived from Lambert showed resistance to both BYDV and WSMV after inoculation in the field. Three of these lines were selected for further testing based of an altered plant phenotype. Transgenic plants inoculated with WSMV showed disease symptoms, however compared to Lambert, plants had lower disease severity ratings, increased survival rates and smaller reductions in plant height. This suggests the pac1 transformants are virus tolerant. More detailed experiments, including assays on virus titer, are required to confirm this.

ERADICATION OF TOBACCO RATTLE VIRUS FROM SOILS BY GROWING WEED-FREE ALFALFA

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Tobacco rattle virus (TRV) causes corky ringspot disease of potato and is transmitted by species of *Trichodorus* and *Paratrichodorus* nematodes. Historically, it has been difficult or impossible to eliminate corky ringspot disease potential from soils once the virus and a nematode vector became established. Soils of the Columbia Basin of Washington in Northwest United States remained free of corky ringspot until recent years, but a major corky ringspot disease epidemic is now underway there. A single nematode vector species, *P. allius*, is present. While monitoring the disease in the Columbia Basin, we observed that TRV could no longer be isolated from soils of a previously heavily contaminated field 3 years after it was seeded to alfalfa (*Medicago sativa*) and maintained essentially weed-free even though the the nematode vector had remained prevalent in the soil. Subsequently, we tested the susceptibility of alfalfa cultivars to TRV in the greenhouse and in field plots, and we measured the retention of TRV in nematodes maintained on alfalfa roots in greenhouse cultures and in the field. Virus detection was based on a reverse transcription-polymerase chain reaction procedure and mechanical inoculation from root tissue to tobacco (*Nicotiana tabacum* cv. Samsun) a host of the virus. TRV could not be detected in the roots of any of eight cultivars of alfalfa at 8 or 16 weeks after seedlings were transplanted into contaminated soils in a greenhouse, but the virus was readily detected in tobacco plants growing in the same soil. Similarly, the virus could not be detected in the roots of four alfalfa cultivars grown in contaminated soil in the field. The virus was detected routinely in tobacco plants grown in the same soil and in potatoes grown in control plots. Nematode vector cultures that initially transmitted TRV to tobacco lost their ability to transmit after maintenance on alfalfa for 3 months in a greenhouse. Subcultures maintained on tobacco retained their capacity to transmit. In a similar field study still underway, the capacity of nematodes in contaminated plots to transmit TRV was reduced 1 year after the plots were seeded to alfalfa. These plots were not completely free of weeds in the first year.

VIRAL DISEASES OF GRAIN CROPS TRANSMITTED BY ARTHROPODS

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In agro-ecological conditions of Ukraine the following viruses of grain crops were identified which are transmitted by arthropods and attack the most important agricultural crops such as winter and spring wheat, barley, millet, oats, sorghum and perennial grasses: wheat streak mosaic virus (WSMV), transmitted by mites (*Aceria tritici* Shev.); wheat mosaic virus (WMV); triticum virus Zachurilo et Sitnikova, transferred by leafhoppers (*Psammotettis striatus* L); bromegrass mosaic virus (BMV), transferred by cereal flea-beetle (*Chaetocema aridula*) and striped grain flea-beetle (*Phyllotreta vitlula*) and beetles and larvae of *Oulema melanopus* L; barley yellow dwarf virus (BYDV), transferred by aphids (*Sitobion avenae* Fabricins and *Toxoptera graminum* Rondani).

Our research has shown that viral infections to reduce the yields of wheat by 12-65 % and degrade the quality of grain (protein content decreased by 2.0 % and gluten content by 4.2-5.5 %). Immunoenzymic analysis (ELISA, sandwich-variant) helped to identify the antigens of WSMV, WMV, BMV and BYDV. Electron microscopy allowed us to detect WSMV, WMV, BMV and BYDV virions. The spread of viral infections on winter wheat crops varied from 5-10% to 60-85% on some plots. WSMV and BYDV are the most widespread in Ukraine.

The aim of our research was to increase the adaptation and other properties of plants which might reduce the degree of infection and ensure higher and more stable yields. A knowledge of the chemical composition of virus-infected plants is helpful in making a correct choice of the methods of optimizing mineral nutrition, to increase the resistance of plants to viral infection. The microelements copper, boron, manganese, zinc, molybdenum and lithium increase crop resistance and yields and improve the quality of grain.

Screening of newly synthesized preparations at The Research Institute of Bio-inorganic and Bio-organic chemistry of the National Academy of Sciences of Ukraine identified certain compounds with antiviral properties. Pre-planting treatment of seed and spraying of wheat plants at the fourth stage of organogenesis with preparations of DG-482 (triman), Agrostimulin and Emistim C, reduced by 1.8 times the number of WSMV-infected plants and considerably decreased the rate of infection, increasing at the same time their dry matter content and the amount of leaf chlorophyl. Above-ground treatment (spraying) of winter wheat plants in field tests increased yield by 9-15 % and improved the quality of grain, increasing the content of protein by 0.9-1.2% and the content of gluten by 1.8-2.5%.

Our research findings have demonstrated varions reactions in plant cells against viral infections. The knowledge of these reactions is helpful in the search for plant protection methods based on the employment of effective and environmentally safe chemical preparations of a direct antiviral action and on the use of bioactive substances which stimulate the cell protective mechanisms. Up-to-date technologies of winter wheat production allow us to use microelements and growth-regulating preparations which increase the immunity of plants against viral infections transmitted by arthropods and do not cause any deterioration of the environment.

INFLUENCE OF APHID POPULATION DYNAMICS ON POTATO VIRUS Y DISSEMINATION IN TOMATO CROPS

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Aphid population dynamics were studied in 1998 using different traps in greenhouses, net and open-air tomato crops in Tenerife Island (Canary Islands) affected by virus potato Y.

The results show that low populations can cause rapid spread. The main species of aphids were: *Aphis gossypii*; *Myzus persicae*; *Macrosiphum euphorbiae*; *Aulacorthum solani*. Infection was less impotant in net greenhouse than in the open-air.

MINI SYMPOSIUM:

LOCAL PROBLEMS RELATED TO INSECT-TRANSMITTED VIRUS DISEASES IN VEGETABLES: EPIDEMIOLOGY AND APPLICATION OF CONTROL STRATEGIES

VIRUS TRANSMITTED BY WHITEFLY: TOMATO YELLOW LEAF CURL VIRUS (TYLCV). CONTROL STRATEGIES

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The geminivirus Tomato yellow leaf curl virus is a *Begomovirus* able to infect several dicot plants including some important crops as tomato and bean. This virus is transmitted by the whitefly *Bemisia tabaci* and it is one of the most important pest of cultivated tomato in many tropical and subtropical regions. During the last 15 years, this virus has been spread widely and today is present in areas of America, Asia, Africa and Europe.

All the isolates of TYLCV reported belong to one of the two known species of this virus: TYLCV-Sa and TYLCV-IL, both of which are present in Spain.

An important effort has been done to develop methods to control the disease produced by TYLCV. Whitely control by chemical treatment has showed to be not efficient enough to avoid the transmission of the virus mediated by the insect. The combination of physical barriers (net), use of insecticides and elimination of infected plants and weeds could partially control the disease in green houses.

A number of projects have been carried out to obtain resistant plants to TYLCV by introducing DNA fragments from virus or related plant species. Breeding and genetic engineering techniques have been used to reach this goal. Up today only partially resistant hybrids are commercially available.

MANAGING WHITEFLIES (*BEMISIA TABACI)*, STRAIN B IN AN AGRICULTURAL SYSTEM

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Sweetpotato whiteflies, *Bemisia tabaci* (Gennadius) strain B, can cause direct crop damage by feeding in plant phloem and removing plant sap that results in reduced vigor. Feeding by nymphs causes plant disorders such as squash silverleaf and irregular ripening of tomato and peppers. Plant disorders, and viral diseases vectored by *B. tabaci* can occur even when a whitefly population is small. Stem blanching, leaf and plant breakdown, chlorosis, yellowing, leaf and fruit shedding and abnormalities of fruiting structures are other forms of damage. Some damage such as leaf and fruit shedding occur only with high whitefly populations that occasionally cause plant death. In cotton, honeydew excreted by whiteflies can cause cotton fiber contamination. Honeydew sugars promote sooty mold that, together with honeydew, reduces fiber quality and make cotton difficult to process. Honeydew contamination can also occur on fruits and vegetables leading to increased production costs.

Whitefly management in a given crop will depend greatly on the relative sensitivity of that crop to infestations of whiteflies. Very few whiteflies are required to transmit viruses provided virus inoculum levels are high. In locations where virus is a major concern and virus sources are present, the grower will want to avoid even small numbers of whiteflies. A combination of selected cultural practices, intensive chemical treatments or physical controls, and/or the development of host plant resistance, may be most effective at managing vector populations. Crops that are not susceptible to viral diseases and are able to tolerate low to moderate populations of whiteflies may be manageable using biological control as the principal control strategy.

Whiteflies in California in recent years have proven to be a year-round problem. The overlapping of whitefly-host crops throughout the annual cropping cycle has encouraged high whitefly densities. As a result of multiple cropping and the extensive use of insecticides, there is a potential for high selection pressure for whitefly insecticide-resistance development. An insecticide resistance management program was instituted in 1993 because of the important role that insecticides played in crop protection strategies against this pest.

Resistance monitoring techniques using yellow sticky cards, hydroponic and plant bioassays treated with formulated insecticides have provided baseline data for a number of pesticides used for whitefly control in California. Continuous areawide monitoring of the whitefly populations for changes in resistance frequencies allowed decisions regarding the formation of effective management strategies. Whitefly insecticide resistance monitoring data and management strategies were communicated to farmers and pest control advisors via newsletters, newspapers, and the Internet. As a result, chemical control has remained effective without the development of detectable resistance.

THRIPS-TRANSMITTED VIRUSES: TOMATO SPOTTED WILT VIRUS. CONTROL STRATEGIES.

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Tomato spotted wilt virus (TSWV) is undoubtedly the most important thrips-transmitted virus due to its high incidence in ornamental and horticultural crops grown in regions with hot climate. *Frankliniella occidentalis* is principally responsible for the dispersal of TSWV in Europe as its populations become very numerous during hot periods and transmission is highly efficient. Transmission is persistent, the larvae of the first phase acquiring the viral particules and the adults being the transmitters; the period of acquisition may be short (some 30 minutes) as occurs with the inoculation period, while the latent period varies according to temperature (176 hours at 20º C and 103 hours at 27º C). In hot regions vector multiplication is permanent and the epidemics of this virosis follow one another all over the year, though the greatest risks exist in spring and autumn, coinciding with the highest population levels of the vector.

The most efficient measures to mitigate the effects of the virosis are those aimed at preventing the epidemics. Reduction of the initial inoculum of the virus and the presence of the vector in the crop, using healthy planting material, is a highly important factor. For this reason there are legal standards of hygiene for nurseries and seed breeding stations where plants are produced. Application of hygienic measures (elimination of alternative hosts of the virus and thrips, elimination of thrips populations in pupal stage in the soil, etc.) before planting the crop are indispensable for a reduction of the initial level of incidence of the disease. Elimination of virus-stricken plants in greenhouse-grown pepper crops reduces virosis incidence to less than a third, even at the most favourable moments for the propagation of the disease. Control of vector populations is an essential element in the control of the epidemics caused by the virus. Specific chemical products are sometimes insufficient when resistance phenomena arise and therefore natural enemies are employed and physical barriers are placed for crop protection. The search for and incorporation of resistance to TSWV into the plant material is a way to permanently resolve the problem caused by this virosis. In the case of tomato and pepper, resistance genes are known in some lines of *Lycopersicon peruvianun* and *Capsicum chinense*, respectively.

CULTURAL CONTROL OF INSECT-TRANSMITTED VIRUSES

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The introduction of the notorious DDT as a commercial insecticide in 1941 heralded an era of pest control dominated by chemical pesticides that persists to this day. This approach created some undesireable side effects including those of pesticide resistance, secondary pest outbreaks and host-plant resistance breakdown. These hazards posed by some aspects of high input agriculture have led scientists to seek more sustainable alternatives. IPM is the solution offered by modern crop protection scientists to overcome these problems. Other important stimuli that are affecting the development of IPM include demands by policy makers for a reduction in usage of pesticides and the requirements of consumers for residue-free food and flowers.

The multidisciplinary approach of IPM is recomended for effective control of virus diseases. Combinations of two or more control measures can result in an improved effect. For example, the use of a vector-killing chemical can complement the planting of a tolerant virus-free certified cultivar.

Non-insecticidal measures have yet to be developed to the stage when they can be recomended to growers as an alternative to conventional pesticide application. Further intensive research efforts are still required to achieve that goal, but the potential and promise of these approaches have been demonstrated by experience in Israel and elsewhere.

RESISTANCE TO APHID-BORNE VIRUSES IN VEGETABLE CROPS

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Aphid-borne viruses constitute a complex pathosystem causing major economical losses to vegetable crops in the Mediterranean basin. Partial virus control can be achieved through cultural practices and prophylactic measures but this is often expensive, time consuming and sometimes applicable to only certain crops or types of growing conditions. In this context, the use of resistant cultivars appears as a simple and cheap approach to limit aphid-borne viruses economical impact.

Breeding for resistance to viruses requires first the identification of resistance genes in germplasm collections. Then, the mechanism and the inheritance of the resistance must be determined. The development of simple and reliable screening procedures will facilitate the rapid breeding of varieties with good agronomic qualities and with the selected resistance character. Resistances to aphid-borne viruses which have been identified in germplasm collections or which are presently available in commercial cultivars will be presented for the major vegetable crops.

Several difficulties may be encountered in the process of breeding for resistance. Sometimes high levels of resistance to one virus are not found. In this case, the association of partial resistances can still lead to an efficient virus control, but is generally a much longer breeding process. Some resistance can be strain specific or easily overcome by new strains of the virus which may evolve from common strains. This may suggest that the resistance will not be durable. Specific studies should then determine the frequency of occurrence (or appearance) of the virulent isolates before considering the use of these resistances in breeding programs. Resistances are often identified in lines with poor agronomic qualities and resistances are inherited concomitantly with one of these poor quality characters. Special efforts are required to try to break these unfavourable 'linkages', particularly when using interspecific crosses. Finally, vegetable crops are generally affected by more than one virus diseases, and breeders prefer developing multi-resistance strategies in order to produce new cultivars resistant to the most frequent viruses.

Due to these difficulties, breeding for resistance using conventional approaches remains a long process. This is probably a reason why virus resistant cultivars are presently so few in vegetable crops. The development of new technologies -such as the production of transgenic plants- and of new concepts -such as the pathogen derived resistance- will probably provide more means to control aphid-borne viruses in the next future.

AUTHORS INDEX

LIST OF REGISTRANTS